Original article:

Age-dependent cardioprotective action of meldonium on heart remodeling under the experimental hypoosmolar hyperhydration

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Abstract

Background: Chronic water overload leads to a remodeling of the internal organs, in particular the heart. The reaction of the organism to the influence of the damaging factors varies depending on age. Therefore, the complex treatment of diseases, which results in a positive water balance, requires the prescription of cardioprotectors.The aim of our study was the determination of morphological changes of the heart of rats of different age under the influence of hypoosomolar hyperhydration and an attempt to correct the detected changes by means of meldonium. ***Material and Method:***The experiment was conducted on 36 laboratory male rats of two age groups: young (3 months old), and old (22 months old).Modeling of severe degree ofhypoosmolarhyperhydration was by the introduction of 10 ml distilled water three times a day and synthetic analogue of antidiuretic hormone «Minirin» (Ferring) 2 times a day at a dose of 0.01 mg without meldonium (first experimental series) and with the parallel introduction of meldonium (second experimental series). We analyzed organometric and histomorphometric data to study the features ofthe heart remodeling. ***Results:*** The organometric study indicated a uniform increase in the weight of the ventricles and a significant dilatation of the right ventricular cavity in young ratsof the first experimental series, and there was a predominant increase in the weight of the right ventricle and uniform dilatation of the cavities of both ventricles in old animals of the same series. The histomorphometric study has determined increasing in the diameter of cardiomyocytes and their nuclei in both age groups. We observed increasing in the relative amount of cardiomyocytes and vessels, and decreasing in relative amount of connective tissue in young rats. In contrast, there was decreasing in relative amount of cardiomyocytes and vessels, and increasing in the relative amount of connective tissue in old animals. The meldonium administration positively acts on the right ventricle, slowing its dilatation in young rats of the second experimental series and its hypertrophy in old rats. Moreover, the diameter of cardiomyocytes in both age groups is significantly increased, but meldonium stabilizes the diameter of the nuclei of cardiomyocytes. Also meldonium increases in the relative amount of vessels in 3-month age rats. ***Conclusions:*** The use of meldonium to correct the morphofunctional state of myocardium in rats under the influence of severe degree of Hypoosmolar hyperhydration significantly reduces the damaging effect of water overload on the myocardium of young animals, and to a lesser extent, on the myocardium of animals of old age. The positive effect of meldonium on the heart of experimental rats is to enhance myocardial vascularization, slowing the growth of the weight of the chambers of the heart, and slowing the dilation of ventricular cavities.

Keywords: heart; cardiomyocytes; hypoosmolarhyperhydration; meldonium

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Introduction

Despite significant progress in treatment and diagnosis, the cardiovascular diseases continue to lead the list of causes of mortality and disability among people of all ages and genders.

According to World Health Organization statistics, cardiovascular diseases take the lives of 17.7 million people every year. That is 31% of all global deaths[1-2].Besidessuch cardiovascular disease risk factors as smoking, physical inactivity, unhealthy diet, overweight, and the harmful use of alcohol,one of the key conditions for normal functioning of the heart is the state of water-electrolyte balance.Positive water balance and hyponatraemia are the result of many pathological conditions. Hyponatremia occurs in about 30% of hospitalized patients. The most common cause of hyponatremia is the syndrome of inappropriate secretion of the antidiuretic hormone (SIADH).SIADH occurs in neurologic disorders, pulmonary infections, HIV infection and acquired immune deficiency syndrome (AIDS), drug use, in particular antidepressants, tumors[3, 4]. In addition, water overload has a city in chronic renal disease, which,in turn, significantly increases the cardiovascular diseases risk[5]. Kidney failure is often an indication for hemodialysis, which only aggravates the hydration[6].Undoubtedly, the body's response to water-salt balance disorders varies according to age[7]. So probably a complex correction of chronic water overload of the body also requires the prescription of cardioprotectors. The efforts of modern experimental and practical medicine are aimed at finding theso-called «ideal» corrector of myocardial metabolism. According to modern concepts[8], «ideal» corrector should protect cellular membrane, preventing the accumulation of fatty acids in cells, activate oxidation of glucose, prevent the formation of lactate, stimulate the oxidation of pyruvate, and reduce oxidative stress. The meldonium meets all these requirements. The action of the meldonium is to reduce the concentration of carnitine, which leads to the restriction of the transport of activated forms of fatty acids into cells. This drug protects the membrane of cardiomyocites, it has antihypoxic, antiarrhythmic, and antithrombotic properties. It is able to expand arterioles and positively affect the contractile function of the myocardium[9]. The aim of our study was the determination of morphological changes of the heart of rats of different age under the influence of hypoosomolar hyperhydration and an attempt to correct the detected changes by means of meldonium.

Material and Method

The experiment was conducted on 36 laboratory male rats of two age groups: young (3 months old), and old (22 months old). The choice of rats as a biological model is due to a number of common features of the structure and function of the cardiovascular system of the rat and humans [10].

Animal care and the experiment itself were conducted in accordance with the requirements of the"European Convention of the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986)and «General ethics of animal experimentation,» approved by the I National Congress on Bioethics (Kyiv, 2001).

All rats were divided into two experimental and one control series.In both experimental series wemodeled a severe degree of hypoosmolar hyperhydration introducing via probe10 ml of distilled water three times a day.Animals were fed by unsaltedfoods and administrated the synthetic analogue of the antidiuretic hormone «Minirin» (Ferring) 0.01 mg together with distilled water 2 times a dayto prevent the physiological support of water homeostasis. Unlike natural arginine-vasopressin, «Minirin» (Ferring) activates only V_2 receptors of vasopressin, which are located in the epithelium of the convoluted tubules and a wideascending part of the Henle's loop. This increases the reabsorption of water in the bloodstream [11]. The simulation of a severe degree of hyperhydration[12]lasted 15 days for young animals and 25 days for mature and old animals.

In addition, the rats of the second experimental series were injected with meldonium intraperitoneally once a day throughout the entire experiment. The dose calculation was carried out according to the formula ofR.S. Rybolovlyev and YU.R. Rybolovlyev [13]:

$$\text{Dose for rat} = \frac{r \times \text{dose for humans}}{R},$$

r – coefficient of species hardiness for a rat = 3,62;

R – coefficient of species hardiness for humans= 0,57.

The animals of control series received normal drinking water and food and were injected the «Mynyrin»(Ferring) twice a day at a dose of 0.01 mg, considering the potential effects of vasopressin on the cardiovascular system.

Experiment results were evaluated using the following methods:

1.Organometry.By the method of G.G. Avtandilov [14], we cut the heart into 4 parts: wall of the left ventricle (LV), wall of the right ventricle(RV), interventricular septum and atria (A). We separately weighed parts

of the heart by W. Muller taking into account the modification of G.I. Ilyin et al.We measured the absolute weight of the LV (LVW) and the RV (RVW) with apart of the interventricular septumroportionally to their weight; the weight of both atrial walls (AW). Also we measured the endocardial surface area of the LV(LVSA) and the RV (RVSA), using indirect planimetry. Moreover, we calculated relative organometric indexes: the ventricular index (VI), which is the ratio of RVW to LVW; and the planimetric index (PI) by the formula LVSA /RVSA.

2. Histomorphometric study of the myocardium.The histological sections, prepared by standard method, were stained with hematoxylin and eosin and investigatedusing a light microscope «OLIMPUS». We measured the diameter of cardiomyocytes (DCMC) of both ventricles and their nuclei (DNCMC), the relative amount of cardiomyocytes (RACMC), connective tissue (RACT), and vessels (RAV)

3.A statistical method. Obtained digital data were processed on a personal computer using the software «GraphPad»[http://graphpad.com]. Reliable probability of error is taken less than or equal to 5% ($p \leq 0.05$).

Ethical clearance: This study was approved by the ethics Committee of Sumy State University, Ukraine

Results and discussion

Under the influence of hypoosmolarhyperhydration there is increase of AW in both age groups. AW is greater than control index by 21.98 % ($p < 0.0001$) in young rats, and by 22.89 % ($p = 0.0001$) in old rats. While the changes in massometric and planimetricventricular parameters in young and old rats vary in different ways.

So, in 3 month age rats of the first experimental series, when they reach a severe degree of hyperhydration, the LVW increases by 28.65 % ($p < 0.0001$), RVW – by 25.77% ($p < 0.0001$)in comparison with the control series. Accordingly, the relative gravimetric index, namely the VI, does not change, indicating a uniform increase in the mass of both ventricles (Table 1). While changes in planimetric indices are characterized by a significant dilation of the cavity of the RV by 18.05 % ($p = 0.0099$), decreasing the PI at 8.98 % ($p < 0.0001$) in comparison with the control series.

There is a statistically significant increase in the mass of both ventricles, which is uneven, with a more rapid hypertrophy of RV in 22-month rats of the first experimental series. The LVW increases by24.48 % ($p < 0.0001$),the RVW – by 45.88 % ($p < 0.0001$), causing the increasing of the VI by 17.27 % ($p < 0.0001$) in comparison with the control series.The dilatation of ventricular cavity occurs evenly: the LVSA increases by 31.35 % ($p < 0.0001$), the RVSA – by 35.12 % ($p < 0.0001$), PI changes statistically unreliable.

Table 1: Relative cardiometric data of control and experimental animals

	3-month age animals			22-month age animals		
	Control series	I experimental series	II experimental series	Control series	I experimental series	II experimental series
VI	0.557 ±0.014	0.532 ±0.008 p= 0.1521	0.533 ± 0.009 p=0.1799	0.388 ± 0.005	0.455 ±0.002 p <0.0001*	0.425 ± 0.002 p <0.0001*
PI	0.802 ±0.05	0.73± 0.006 p< 0.0001*	0.763 ± 0.015 p=0.4722	0.901 ± 0.008	0.88 ±0.006 p=0.0621	0.902± 0.005 p=0.9177

(Comparison between control and experiment data done by unpaired Student's 't' test, * = statistically significant).

Such divergences of ventricular remodeling are due to anatomical and functional features: LV works as a pressure pump, and RV – as volumetric [15, 16]. Inaddition, reducing the oncotic pressure of blood plasma causes swelling of the interstitium of the lungs, compressing the pulmonary vessels. It leads to hypertension in the lesser circulation and to increase the mass of RV [17, 18, 19], causing compensatory hypertrophy of the RV. The LV hypertrophy is due an increase in the volume of the circulating fluid. The rising volume of the circulating fluid causes the expansion of atrial wall, thereby activating the secretion of the atrial natriuretic peptide. The level of atrial natriuretic peptide positively correlates with progression of dilatation of the RV[20]. In addition, it has been proved that the RV accumulates fluid much faster than LV[21, 22].

Since RVW in young rats is relatively higher than that in old animals, RV in young rats is more adapted to overcome overloads. A rapid increase in LVW

is a physiological feature of this age category and indicates significant compensatory opportunities of LVin young animals.Significant expansion of the LV in old rats suggests disadaptation processes due to age and severity of experimental animals as well as the development of stagnation in the greater circulation.

Hystomorphometrically,a significant increase in the DCMC is observed.There is increasing of the DCMC of the LV by 12.37 % ($p < 0.0005$),DCMC of the RV by 11.1 % ($p < 0.0001$)in young animals, in old rats – by 15.44 % ($p < 0.0001$) and 15.21 % ($p < 0.0001$) respectively (Table 2). DNCMC of LV and RV in young rats is greater than control index by 3.95 % ($p = 0.0095$) and 4.78 % ($p = 0.0493$) and by 6.1 % ($p = 0.0401$) and by 5.05 % ($p = 0.0442$) in old rats. Thus, DCMC of both ventricles grows much faster than DNCMC. Such dynamics of morphometric indices can be considered as a compensatory reaction to the load of the ventricles of the heart and a sign of hypertrophy [23].

Prolonged water overload increases the permeability of cell membranes and, according to the osmolarity gradient, the fluid from the intercellular space enters the cardiomyocytes, causing their edema. It leads to increasing ofRACMC of the LV by0.56 % ($p = 0.0218$), increasing of RAV of the LV by 1.48% ($p = 0.05$) and decreasing in RACT of the LV at 7.25 % ($p = 0.0002$)in comparison with the control in young rats. The RACMC is less than control index by 0.39 % ($p = 0.0357$), RAV increases by 1.78 % ($p = 0.0049$), and the RACTchanges unreliable in myocardium of the RV inyoung rats (Table 2). The obtained data indicate the predominance of cellular edema in the LV myocardium and stromal edema in the RV myocardium. The increase in the RAV of both ventricles suggests significant reserve capacity of the myocardium of rats in this age group. Increasing in the RAV improves myocardial trophy of young animals, and is a sign of activation of compensatory and adaptive mechanisms [24].

In old rats of the first experimental series the RACMC of the LV and RV are less than control data by0.58 % ($p = 0.0115$) and 0.89 % ($p = 0.0008$) respectively. Also there is decreasing in RAV of the LV by 2.22 % ($p = 0.0500$) and RAV of the RV by 2.69 % ($p = 0.0384$). RACT of the LV is greater than the control index by 5.18 % ($p < 0.0001$), and RACT of the RV– by 7.47 % ($p < 0.0001$).

Table 2: Hystomorphometric data of control and experimental animals

	3-month age animals			22-month age animals		
	Control series	I experimental series	II experi-mental series	Control series	I experimental series	II experi-mental series
DCMC LV,mkm	10.19 ± 0.05	11.92 ± 0.1 p<0.0001*	11.45 ± 0.09 p<0.0001*	16.32 ± 0.13	18.84 ± 0.25 p <0.0001 *	18.47 ± 0.17 p<0.0001*
DNCMCLV, mkm	4.05 ± 0.03	4.21 ± 0.04 p=0.0095*	4.14 ± 0.05 p=0.1537	5.57 ± 0.08	5.91 ± 0.16 p=0.0401*	5.81 ± 0.14 p=0.1675
DCMCRV, mkm	10.45 ± 0.05	11.941 ± 0.08 p<0.0001*	11.61 ± 0.07 p<0.0001*	15.58 ± 0.13	17.95 ± 0.23 p <0.0001*	17.49 ± 0.19 p<0.0001*
DNCMC RV, mkm	4.18 ± 0.04	4.38± 0.08 p=0.0493*	4.26 ± 0.05 p=0.2400	5.74 ± 0.07	6.04 ±0.13 p = 0.0442*	5.91± 0.11 p=0.2215
RACMCLV, %	85.08 ± 0.12	85.56± 0.13 p=0.0218*	85.29 ± 0.1 p=0.2085	83.37±0.11	82.89 ± 0.11 p=0.0115*	83.04 ± 0.14 p=0.0935
RAV LV, %	7.41± 0.02	7.53±0.05 p=0.05*	7.53±0.04 p=0.0230*	5.28±0.05	5.16±0.02 p=0.0500*	5.19± 0.03 p= 0.1537
RACT LV %	7.45± 0.05	6.91 ± 0.08 p= 0.0002 *	7.18 ± 0.06 p=0.0062 *	11.35±0.05	11.95 ±0.09 p <0.0001*	11.77 ± 0.11 p=0.0060*
RACMCRV, %	85.26 ± 0.08	84.93 ± 0.11p=0.0357*	84.92 ± 0.08 p=0.0132*	82.98±0.09	82.234 ± 0.13 p =0.0008*	82.46 ± 0.08 p=0.0015*
RAV RV, %	7.32 ± 0.03	7.45 ± 0.05 p=0.0499*	7.43 ± 0.02 p=0.0122*	5.17±0.03	5.031 ± 0.05p =0.0384*	5.07 ± 0,04 p=0.0734
RACT RV,%	7.42 ± 0.05	7.62 ± 0.09 p=0.0807	7.65 ± 0.06 p=0.0147*	11.85 ± 0.06	12.735±0.08 p <0.0001*	12.47 ± 0.05 p<0.0001*

(Comparison between control and experiment data done by unpaired Student's 't' test, * = statistically significant).

The decreasing in RAV of both ventricles myocardium is associated with a decrease in the compensatory capacity of the myocardium with age [25]. The intracellular edema is more pronounced in young animals, and stromal – in rats of senile age. This is due to the uneven physiological distribution of water[26]: the prevalence of its content is intracellular in young individuals and extracellular in - old.

On the background of meldonium introduction, the significant increase in the mass of all heart`s chambers is maintainedin young animals of the second experimental series. The LVW increases by 23.03% ($p < 0.0001$), RVW - by 20.1% ($p < 0.0001$), AW - by 14.29% ($p = 0.0003$). At the same time, changes in VI do not occur, and PI is not as rapidly as in animals that did not receive a meldonium (Figure 1).

The most pronounced changes in organometric parameters of the heart are observed in old animals of the second experimental series: the LVW increases by 25.45 % ($p < 0.0001$), RVW – by 37.28 % ($p < 0.0001$), AW – by 19.88 % ($p = 0.0002$). Changes in relative gravimetric indices indicate an overwhelming RV hypertrophy, but its degree is lower than in animals that have not received a meldonium.Thus, VI increases by 9.54% ($p < 0.0001$) in comparison with a control series. There is revealed proportional dilation of cavities of the left and right ventricles: the LVSA increases by 22.77 % ($p = 0.0013$), and RVSA – by 22.68 % ($p = 0.0012$) (Figure 2).

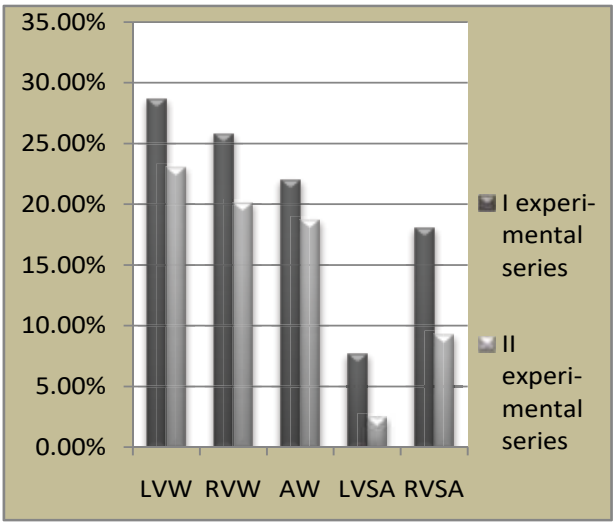

Figure 1. Changes in cardiometric data of 3-month-old rats of both experimental series in comparison with the control

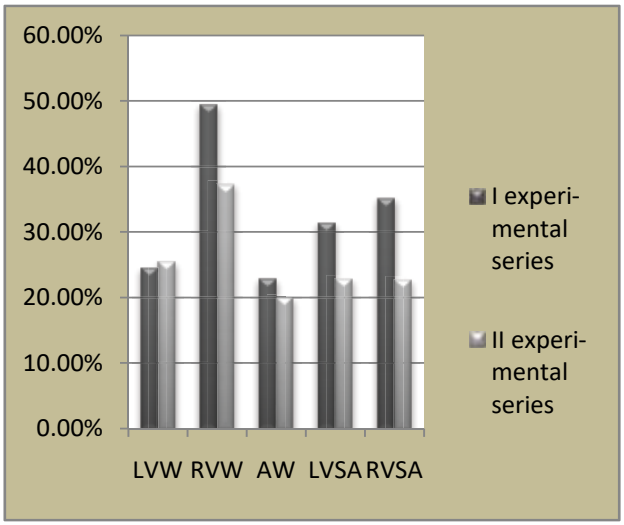

Figure 2.Changes in cardiometric data of 22-month-old rats of both experimental series in comparison with the control

Hystomorphometrically, a significant increase in the DCMC is observed, but the impairment of the tissue ratio of the myocardium in both age groups is significantly less, than in the first experimental series. Thus, in young animals there is increasing of the DCMCof the LV by12.37 % ($p < 0.0005$) and DCMC of the RV by 11.1 % ($p < 0.0001$). While, the DNCMC of both ventricles varies statistically unreliable in comparison with the control series.RACT in LV decreases by 3.62 % ($p = 0.0062$), andRACT in RV – by 3.1 % ($p = 0.0147$),RAV in the left and right ventricles increases by 1.62 % ($p = 0.0230$) and by 1.5 % ($p = 0.0122$) respectively (Figure 3).

Conclusions

The use of meldonium in rats under the influence of severe degree of hypoosomolar hyperhydration to correct the morphofunctional state of myocardium significanantly reduces the damaging effect of water overload on the myocardium of young animals, and to a lesser extent, on the myocardium of animals of old age. The positive effect of medonium on the heart of experimental rats is to enhance myocardial vascularization, slowing the growth of the weight of the chambers of the heart, and slowing the dilation of ventricular cavities.

Conflict of interest statement: We declare that we have no conflict of interest.

Authors' Contribution:

Data gathering and idea owner of this study: Olga Y, Vitaliy S, Valentyna B, Olga P,

Study design: Olga Y, Vitaliy S, Valentyna B, Olga P, Nadiia D, Lina B

Data gathering: Olga Y, Vitaliy S, Valentyna B

Writing and submitting manuscript: Olga Y, Vitaliy S, Nadiia D

Editing and approval of final draft: Olga Y, Olga P, Lina B

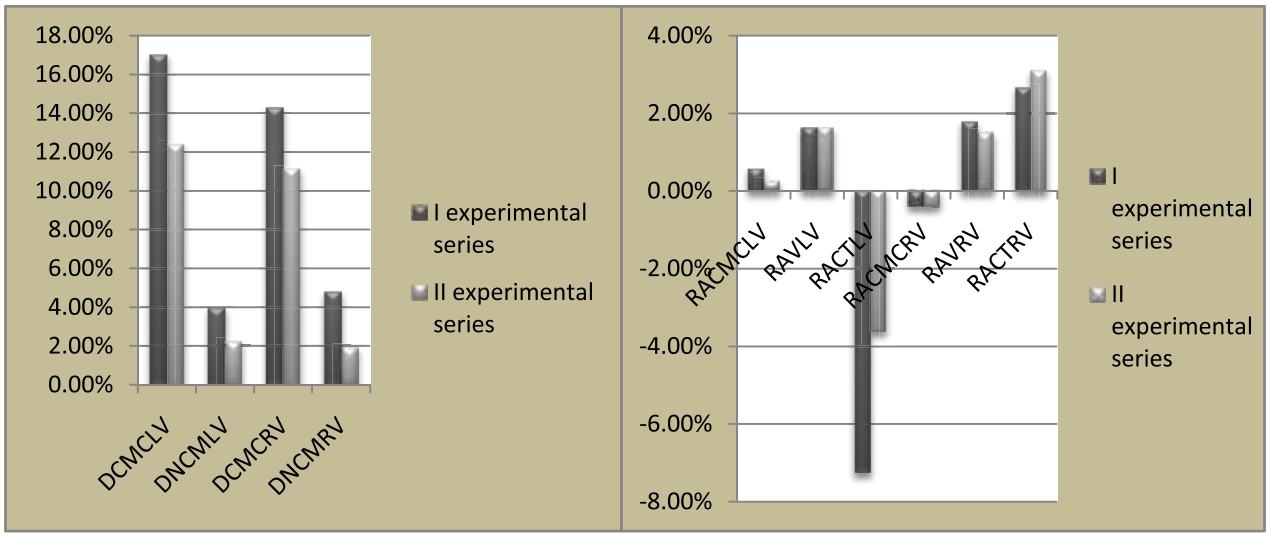

Figure 3.Changes in hystomorphometrical data of 3-month-old rats of both experimental series in comparison with the control In old rats of the second experimental series the DCMC is more than control data by 13.17 % ($p < 0.0001$) in LV and by 12.26 % ($p < 0.0001$) in RV. RACMC in RV is less than control by 0.62 % ($p = 0.0015$). RACTincreases by 5.29 % ($p < 0.0001$) in LV and by 5.23 % ($p < 0.0001$) in the RV(Figure 4).

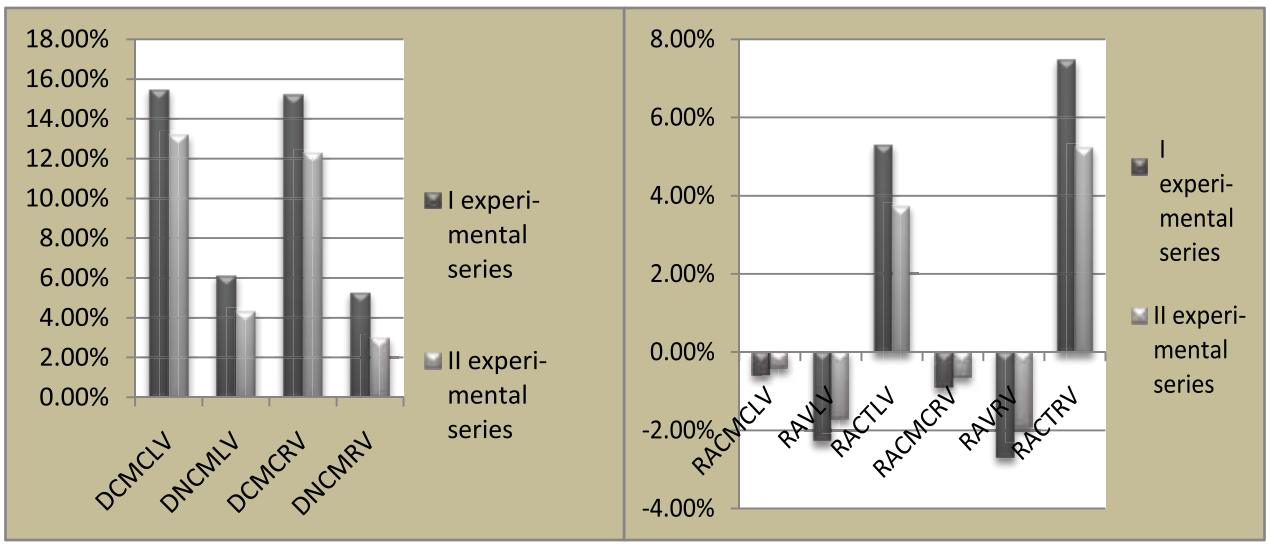

Figure 4.Changes in hystomorphometrical data of 22-month-old rats of both experimental series in comparison with the control

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