

## The importance of aquatic plants in maintaining zooplankton diversity and abundance: An experimental approach

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The influence of different species and ecological forms of higher aquatic plants on the abundance, biomass and species diversity of zooplankton was investigated in experimental ecosystems (microcosms). It was shown that in the absence of fish and macroinvertebrate predators, the abundance and diversity of zooplankton in the plants beds increased. Not only phytophilous, littoral, and small planktonic crustaceans, but also some large obligate planktonic crustaceans, reached a high abundance among the plants. Plants belonging to different ecological groups stimulated an increase in the abundance of different groups of zooplankton. In the beds of submerged plants, the number of Cladocera increased to a greater extent than Copepoda or Rotifera. The abundance of Copepoda, both Cyclopoida and Calanoida, grew in the beds of helophytes. The relative number of predators in the community increased among the plants. The species structure and quantitative parameters of zooplankton had their own characteristics in monospecies and mixed phytocenoses of the same plant species. The index of species diversity of zooplankton acquired the highest values in the mixed phytocenosis. Higher aquatic plants increase spatial heterogeneity, which stimulates the development of facultative planktonic and benthic species. The formation of phytogenic detritus and the lifetime release of organic substances by plants into the water causes the development of bacteria and protozoa, which expands the quality and size range of food organisms for zooplankton. Therefore, zooplankton becomes more diverse in terms of the set of trophic groups.

**Keywords:** aquatic plant; Cladocera; Copepoda; biodiversity; microcosms.

### Introduction

Higher plants are an important component of aquatic ecosystems. As autotrophs, plants are involved in the processes of production and destruction of organic matter, and largely determine the functioning of ecosystems. The structural organization of space, hydrodynamics, hydrochemical indicators, illumination, the degree of sedimentation of suspended solids and agitation of sediments vary depending on the area and time of littoral overgrowth by plants. Plants affect aquatic and near-water organisms of various systematic groups (Dvořák, 1987). Researchers often note a higher abundance and species diversity of zooplankton in the beds of aquatic plants than in open areas (Meerhoff et al., 2007; Semenchenko & Razlutsky, 2009; Kuczyńska-Kippen & Joniak, 2010). However, the effect of plants on zooplankton can be either positive or negative. On the one hand, favourable conditions for the zooplankton are created among aquatic plants due to less mixing of water masses and a richer food base thanks to phytogenic detritus and microorganisms on it. In this case, the beds of plants can act as a kind of reserve for the restoration of abundance and biological diversity. On the other hand, the morphology of plants, their belonging to a certain ecological group, and the density of beds affect the structure of space. Sometimes changes occur in such a way that not all forms of zooplankton (in particular, obligate planktonic species) can fully develop, experiencing restrictions in movement and, as a result, in feeding. In addition, higher aquatic plants change the structure of phytoplankton, competing with algae for nutrients and light (Mjelde & Faafeng, 1997). For some plants, allelopathic activity is shown in relation to certain groups of algae (van Donk & van de Bund, 2002). Therefore, species of zooplankton that feed on algae and have a high selectivity of food items may not find enough food among plants. One of the important factors determining the distribution of zooplankton in water bodies is the influence of

predators. It is often noted that planktonic organisms take shelter among beds of plants, avoiding being eaten by fish, and then their abundance in the overgrown littoral increases. At the same time, significant pressure from invertebrate predators is possible in the beds (Meerhoff et al., 2007), leading to a decrease in the number of some species.

In the course of field studies, it is difficult to establish to what extent exactly plants, as an environment-forming factor, affect zooplankton. In water bodies, zooplankton are exposed to a whole range of factors. One of the most significant is the predation of fish and macroinvertebrates. The direct effect of plants on zooplankton can be distinguished in experiments with model ecosystems (microcosms), when most conditions are created and controlled by the researcher. The purpose of the work is to experimentally identify changes in the abundance, biomass and diversity of zooplankton in the beds of higher aquatic plants from different ecological groups.

### Material and methods

The study was carried out on the basis of the experimental field and expeditionary work station of the Institute for Biology of Inland Waters Russian Academy of Sciences (58°02' N, 38°14' E). Microcosms were created in plastic trays 1 x 1 x 0.5 m, which were filled with river water filtered through a sieve with a mesh of 63 µm to a volume of 300 L. Algae and microorganisms fell into the trays with water. Zooplankton were captured separately in ponds, concentrated in one tank, and then distributed in equal amounts into the trays. We created Lefler microcosms “mixed flask culture” (Lefler, 1984), which are artificial ecosystems that initially contain organisms that are present in a natural water body, the number and composition of which is determined by chance, and not by the experimenter. Microcosms were placed in the open air under natural

light in a pool filled with water to avoid sharp daily temperature fluctuations. Trays were covered with mosquito nets to prevent uncontrolled entry of insects, mollusks and other organisms into microcosms. Microcosms without higher aquatic plants served as a control. Plants belonging to various ecological groups (submerged, floating and emergent) were placed in experimental trays. Each treatment was performed in triplicate. The effect of five species of aquatic plants taken from nature on the abundance and diversity of zooplankton was studied in two experiments. Densities of the beds of plant corresponded to natural. Observations were carried out for two months during the period of active vegetation of plants. The first experiment took place from July 2 to September 2, 2008 and included treatments: the control (without plants), with submerged hydrophytes – homwort *Ceratophyllum demersum* L. (3.3 g/L) and Canadian pondweed *Elodea canadensis* Michx. (3.3 g/L), as well as a treatment with *Hydrocharis morsus-ranae* L. floating on the surface (250 plants per microcosm). The second experiment lasted from July 1 to August 29, 2010. It included the treatment with submerged water soldier *Stratiotes aloides* L. (10 plants per microcosm), which differ from homwort and elodea by shape, and the treatment with helophyte arrowhead *Sagittaria sagittifolia* L. (20 plants per microcosm). In addition, there was the treatment with mixed phytocenosis, when in microcosm both water soldier (5 plants) and arrowhead (10 plants) were placed. Arrowhead was rooted in basins with soil. To create equal conditions in the microcosms of the second experiment, basins with soil were installed in the microcosms of all treatments, including the control.

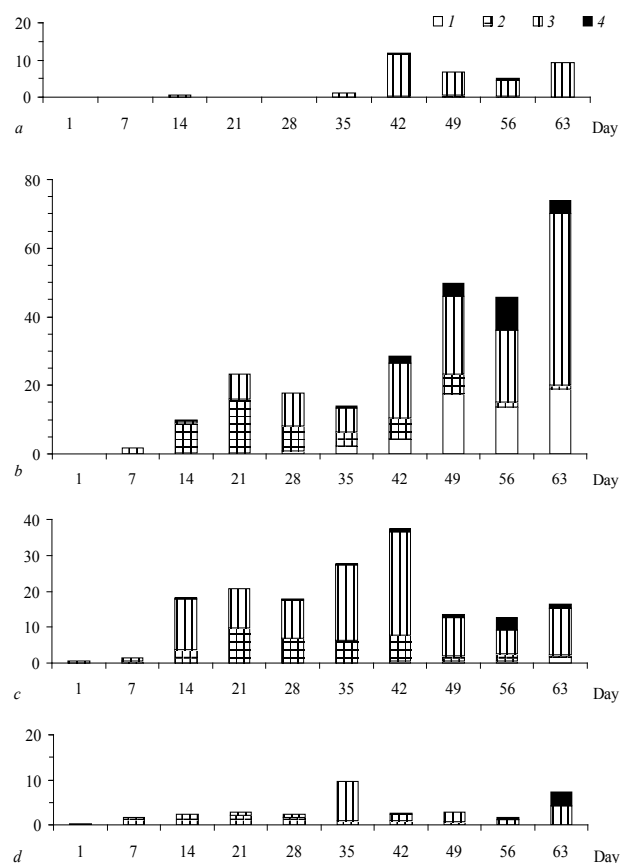
Zooplankton for analysis was selected every 7 days in the first experiment and every 5 days in the second one. Samples were taken at six points of each microcosm with a 0.5 L sampler (3 L total). Zooplankton was concentrated by filtering through a plankton net and fixed in 4% formalin. Zooplankton was counted and analyzed using methods generally accepted in hydrobiology. The species composition of zooplankton, the abundance and biomass of individual species and major groups (Rotifera, Cladocera, and Copepoda) were determined, and the Shannon index of species diversity and the Sørensen index of species similarity were calculated. Taking into account the small number of replicates, nonparametric methods of statistical analysis were used. The differences in indicators between the treatments were evaluated using repeated measures ANOVA (Friedman test). Pairwise comparisons were made using the Wilcoxon test with Bonferroni correction. Calculations were performed in the PAST program (Hammer et al., 2001).

## Results

In the first experiment, the total number of zooplankton in the microcosms with submerged plants (elodea and homwort) began to exceed the control values from the second week of observations. A significant difference in the abundance of Cladocera was observed in the experimental ecosystems with both submerged plants (Fig. 1). In the beds of homwort, additionally, the number of Copepoda also increased (Table 1) largely due to the juvenile stages of Cyclopoida. The differences between the zooplankton of the phytocenoses of the two submerged plant species were in the higher number of Copepoda (Friedman test:  $P < 0.05$ ) and Rotifera ( $P < 0.01$ ) in the homwort beds and the higher biomass of Cladocera ( $P < 0.05$ ) produced by large-sized *Daphnia longispina* O. F. Müller and *Simocephalus vetulus* (O. F. Müller) in the Canadian pondweed beds.

The abundance of zooplankton in the microcosms with floating *Hydrocharis morsus-ranae* did not differ from the control during the first month of observation. Then the abundance of zooplankton in the control increased, but the abundance of zooplankton in the beds of *Hydrocharis* remained at the same level (~ 24 ind./L). Significantly lower numbers of Cladocera and Copepoda were recorded in the microcosms with *Hydrocharis* than with both types of submerged plants ( $P < 0.001$ ). *Daphnia longispina*, *Simocephalus vetulus* and Chydoridae abundantly developed in the beds of homwort and Canadian pondweed, but these crustaceans were few or absent in microcosms with *Hydrocharis*. However, the predatory calanoid *Heterocope saliens* Lilljeborg, as in the control, was found in samples for a longer period (6 weeks) than with submerged plants. In the beds of Canadian pondweed, *H. saliens* disappeared from samples after two weeks and after 4 weeks in the beds of homwort. Rotifera were in

small numbers everywhere. The number of Rotifera species found in the beds of homwort and *Hydrocharis* exceeded the control (Table 1).



**Fig. 1.** Abundance (ind./L) of Cladocera species in the experiment 1: *a* – control, *b* – in the treatment with *Elodea canadensis*, *c* – in the treatment with *Ceratophyllum demersum* and *d* – in the treatment with *Hydrocharis morsus-ranae*; 1 – *Daphnia longispina*, 2 – *Simocephalus vetulus*, 3 – Chydoridae, 4 – other Cladocera

Indices of species diversity calculated by both abundance and biomass in the microcosms with all plant species increased in comparison with the control (Table 1).

The highest index of zooplankton species similarity was found in the microcosms with plants belonging to the same ecological group. The species similarity of zooplankton in the beds of homwort and Canadian pondweed was 67.7%. A small similarity in the zooplankton species composition was found between the control and the microcosms with *Hydrocharis* (42.3%) and between the control and the microcosms with Canadian pondweed (43.4%). The species similarity of zooplankton developing in trays with *Hydrocharis* with that formed among submerged plants was 54.0%.

In the second experiment, the abundance of zooplankton increased in all experimental ecosystems with plants. On average, during the experiment, the abundance of zooplankton in the water soldier beds was higher than in the control by 23.4%, in the arrowhead beds by 67.4%, and in the mixed phytocenosis by 29.4%. Aquatic plants belonging to different ecological groups caused the development of different zooplankton groups. In the beds of submerged water soldier, a greater abundance of Cladocera was recorded, which also provided a larger biomass (Table 2). Copepoda developed massively in the beds of helophyte arrowhead, which, due to small-sized juvenile individuals, significantly increased the abundance, but not the total biomass of zooplankton.

In the experiment, the average index of species diversity in the microcosms with plants exceeded the control. Significant differences were obtained between control and the treatment with water soldier when calculating in numbers, as well as between the control and the treatment with arrowhead when calculating in biomass. In the mixed phytocenosis, the reliability of differences with the control was found in both cases of calculation (Table 2).

**Table 1**  
The indicators of zooplankton in the experiment 1

Indicator	Treatment			
	control	<i>Ceratophyllum</i>	<i>Elodea</i>	<i>Hydrocharis</i>
Abundance of Rotifera, ind/L	0.5 (0–2)	0.8 (0–4)	0.4 (0–1)	1.3* (0–7)
Abundance of Cladocera, ind/L	0.9 (0–12)	17.1* (1–38)	20.5* (0–74)	2.6 (0–10)
Abundance of Copepoda, ind/L	20.1 (6–118)	125.4* (5–271)	74.8 (8–143)	18.1 (8–30)
Biomass of Rotifera, mg/L	0.0008 (0.0002–0.0021)	0.0008 (0.0001–0.003)	0.0003 (0–0.0011)	0.0014 (0–0.0064)
Biomass of Cladocera, mg/L	0.0063 (0–0.0797)	0.2528* (0.0046–0.7751)	0.7045* (0.146–1.6981)	0.1298* (0.01–0.214)
Biomass of Copepoda, mg/L	0.4335 (0.116–1.5032)	0.3788 (0.1918–1.797)	0.4036 (0.2736–2.2273)	0.5295 (0.0746–3.9668)
Shannon index bit/ind.	1.0 (0.3–2)	2.4* (1–2.9)	2.0* (0.6–2.8)	2.6* (0.1–3)
Shannon index bit/mg	0.5 (0.0–2.5)	1.9* (0.1–2.5)	1.8* (0.3–2.2)	1.7* (0.0–2.2)
Number of Rotifera species	10	13	9	14
Number of Cladocera species	7	9	10	7
Number of Copepoda species	8	9	10	9

Note: the median, minimum, and maximum values are specified for abundance and biomass; asterisks indicate indicators that are significantly different from the control (Friedman test,  $P < 0.05$ ).

**Table 2**  
The indicators of zooplankton in the experiment 2

Indicator	Treatment			
	control	<i>Stratiotes</i>	<i>Sagittaria</i>	<i>Stratiotes + Sagittaria</i>
Abundance of Rotifera, ind/L	0.3 (0–4)	1.2 (0–4)	1.2 (0–3)	0.9 (0–5)
Abundance of Cladocera, ind/L	31.8 (11–83)	50.0* (25–84)	42.5 (11–84)	37.2 (13–61)
Abundance of Copepoda, ind/L	27.3 (15–54)	30.4 (15–44)	60.4* (17–145)	55.1* (14–95)
Biomass of Rotifera, mg/L	0.0003 (0.0–0.0024)	0.0008* (0.0002–0.0027)	0.0007 (0.0001–0.0024)	0.0007 (0.0001–0.0037)
Biomass of Cladocera, mg/L	1.3235 (0.16–5.29)	2.2508* (1.04–4.57)	1.1614 (0.22–2.48)	0.6750* (0.18–3.2)
Biomass of Copepoda, mg/L	0.2660 (0.17–0.76)	0.1958 (0.08–0.37)	0.5843* (0.28–1.15)	0.3483 (0.11–0.81)
Shannon index bit/ind.	1.0 (0.7–1.5)	1.3* (0.8–1.7)	1.1 (0.7–1.4)	1.4* (0.9–1.6)
Shannon index bit/mg	0.8 (0.4–1.6)	0.8 (0.4–1.9)	1.3* (0.6–1.8)	1.6* (0.6–2.1)
Number of Rotifera species	13	22	18	23
Number of Cladocera species	13	14	13	14
Number of Copepoda species	11	9	11	11

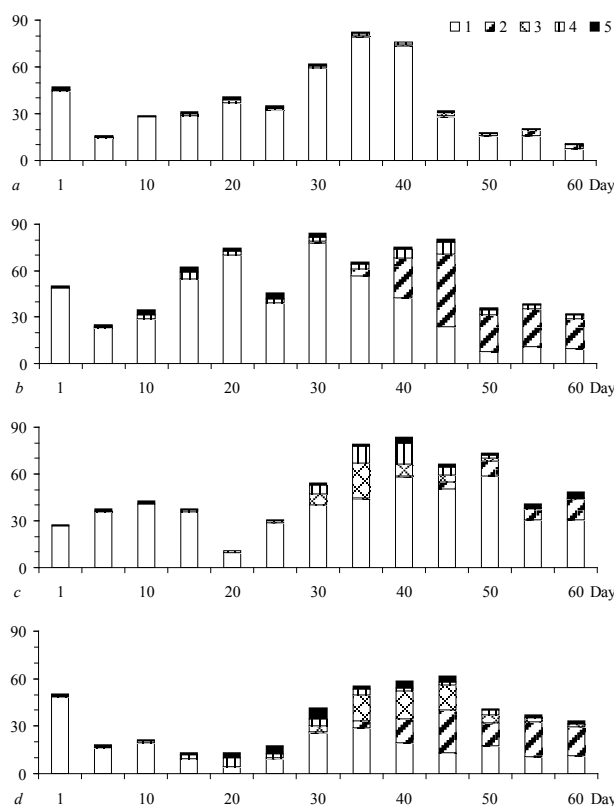
Note: see Table 1.

The species similarity of zooplankton between the control and the arrowhead beds was 65.5%, between the control and the treatment with water soldier it was 56.1%, between the control and the mixed phytocenosis it was 58.2%. Zooplankton from the microcosms with different types of plants in the species composition coincided by 61.9%. The highest rates of zooplankton species similarity were observed between the treatments: mixed phytocenosis – water soldier (74.4%) and mixed phytocenosis – arrowhead (70.2%).

The planktonic filter-feeder *Daphnia longispina* was the dominant species in the control communities (Fig. 2). Water soldier did not affect the development of *Daphnia*. The abundance and biomass of this species remained similar to the control values (an average of 38 ind/L). At the same time, the number of other large filter-feeder calanoid copepod *Eudiatomus transylvanicus* (Daddy) decreased by half. In the second half of the experiment, the number of *Ceriodaphnia quadrangula* (O. F. Müller) increased (up to 47 ind/L) in the water soldier beds. Only the microcosms with water soldier were characterized by an increase in the abundance of this species.

In the experimental ecosystems with arrowhead, the proportion of Cladocera decreased relative to the control values. *Daphnia longispina* developed with a smaller population. But the conditions in the arrowhead beds were favourable for the growth and reproduction of the microfilter *Diaphanosoma brachyurum* (Liévin). *Diaphanosoma* was not found in the beds of water soldier. Also, only in the treatment with the arrowhead, the rotifer-microphage *Filinia longiseta* (Ehrenberg) was noted in the samples. Copepoda abundance and biomass exceeded these indicators in other treatments (Table 2). The number of both Cyclopoida of all age stages and Diaptomidae has increased.

In the mixed phytocenosis, the number of *Daphnia* decreased to the greatest extent, which resulted in a significantly lower biomass of cladocerans in total. However, there was an abundance of both *Ceriodaphnia quadrangula* (as with only water soldier) and *Diaphanosoma brachyurum* (as with only arrowhead). The number of Calanoida remained similar to the control values. The number of Cyclopoida grew more than with a single arrowhead.



**Fig. 2.** Abundance (ind/L) of Cladocera species in the experiment 2:  
a – control, b – in the treatment with *Stratiotes aloides*, c – in the treatment with *Sagittaria sagittifolia*, d – in the mixed phytocenosis; 1 – *Daphnia longispina*, 2 – *Ceriodaphnia quadrangula*, 3 – *Diaphanosoma brachyurum*, 4 – Chydoridae and 5 – other Cladocera

The index of zooplankton species diversity acquired the highest values when two plant species were present simultaneously. The maximum relative number of predators was observed in the mixed phytocenosis. Predators accounted for 3.9% of the population and 9.0% of the zooplankton biomass, while they were only 1.0% of the population and 1.6% of the biomass in the control. According to the results of both experiments, all microcosms with plants were characterized by an increase in the proportion of predators in zooplankton ( $P < 0.001$  for microcosms with Canadian pondweed, water soldier, arrowhead, and mixed phytocenosis;  $P < 0.01$  for microcosms with *Hydrocharis* and hornwort).

In both experiments, in the aquatic plants beds, with the exception of the treatment with *Hydrocharis*, the abundance of crustaceans from the family Chydoridae significantly exceeded the control parameters (with hornwort, Canadian pondweed, and water soldier  $P < 0.001$ , with arrowhead, and in the mixed phytocenosis  $P < 0.01$ ).

## Discussion

In the conducted experiments, the influence of fish and predatory macroinvertebrates were excluded. All changes in zooplankton were determined by plants. The diversity of zooplankton increased in all microcosms with plants. The abundance of crustaceans also increased in the experimental beds of plants, with the exception of the microcosms with floating *Hydrocharis morsus-ranae*. Apparently, in the latter case, the predatory pressure of *Heterocope saliens* on other zooplankton was palpable (Kurbatova & Ershov, 2012).

One of the significant changes in the habitat caused by aquatic plants is the complexity of the structural organization of space. It is most evident in the beds of submerged plants which entirely develop in the water column. The appearance of large areas of underwater substrates encourages the development of facultative planktonic and benthic species. An increase in the abundance and diversity of crustaceans Chydoridae was associated with this factor in most microcosms with plants.

The reactions of different groups of zooplankton in the experiments are consistent with the results of the study, when artificial plants of various forms were placed in natural water bodies. Cladocera and Cyclopoida reached the highest numbers among plastic plants imitating submerged species, smaller numbers in floating ones, and even smaller in the open water zone. Calanoida preferred open habitats (Meerhoff et al., 2007). In our microcosms, the floating *Hydrocharis* did not affect the population dynamics of the predatory Calanoida *Heterocope saliens*, but in the beds of submerged plants, the number of this crustacean declined rapidly. In another experiment the filter-feeder Calanoida *Eudiaptomus transylvanicus* decreased in number in the treatment with submerged water soldier also. However, the development of *E. transylvanicus* was stimulated in the beds of helophyte arrowhead, whose smooth leaf petioles and peduncles occupied a small volume in the water column. Regardless of the type of food, calanoids need open spaces to move freely and provide themselves with food. Dense thickets of submerged plants create a spatial organization of the environment that is unsuitable for Calanoida.

Probably, mechanical reasons largely explain the avoidance by other large obligate planktonic crustaceans of beds of higher aquatic plants. It was shown that *Daphnia*, in the absence of a signal about predators, equally ignored both zones with real plants and their artificial analogues (Lauridsen & Lodge, 1996; Tavşanoğlu et al., 2012). In the experiment with artificial plants imitating the submerged hydrophyte *Myriophyllum* (Serbin et al., 2007), *Daphnia magna* Straus individuals reached smaller sizes than in the control and produced fewer eggs of a larger size. The authors believe that the negative effect was caused by two factors: the rapid settling of food particles on the surface of plants and the high energy consumption of *Daphnia* to “bypass” obstacles in a complex environment. On the contrary our experiments revealed the possibility of abundant development of *Daphnia* among living submerged plants (in the treatment with *Elodea* in the first experiment and in the treatment with water soldier in the second one). Other researchers also described a situation when, in the absence of predators, the number of *Daphnia* in the beds of hydrophytes exceeded that in the plant-free zone (Jacobsen et al., 1997). With a small or moderate density of phytocenoses, the richness of the food base among plants becomes a decisive factor for the formation of zooplankton.

Studies of the pelagial seston and the overgrown littoral of four lakes found no differences in quantitative indicators between the two zones. But in terms of N/C, P/C, and polyunsaturated fatty acid content, seston was of the best quality among hydrophytes (Booker & Cheruvilil, 2011).

Higher aquatic plants affect one of the main groups of food objects of zooplankton filtrators – algae. Often, due to competition and sometimes allelopathy, plants change the species and size structure of phytoplankton (Mjelde & Faafeng, 1997; van Donk & van de Bund, 2002) and its abundance in such a way that this type of food is not enough for zooplanktons. At the same time, the importance of other forage organisms grows among plants. A large amount of detritus is formed in biotopes inhabited by plants, and researchers note a high number of bacteria (Rybakova, 2010). Naturally, the turnover of organic matter is activated through the “microbial loop”. As a result, the number of flagellates and infusoria increases. Protozoa and bacteria supplement the diet of planktonic filtrators, particularly *Daphnia* (Carrick et al., 1991; Sanders et al., 1996). It is indicated that protozoa are an important food source for zooplankton at low phytoplankton abundance (Sanders & Wickham, 1993). Obviously, a sufficient amount of available food allowed *Daphnia* to create a significant number and biomass among plants in the experiments.

The expansion of the qualitative and dimensional spectrum of food objects leads to the possibility of increasing the diversity of zooplankton. Both grazing and detritus food chains are realized in the plant beds. The functioning of the “microbial loop” allows microfiltrators to develop (*Diaphanosoma brachyurum* and *Filinia longiseta* appeared in the microcosms with arrowhead) and contributes to the growth of the number of predators (young Cyclopoida can grow by feeding on infusoria).

In the process of life, plants can significantly change the hydrochemical parameters of the environment. As a result of active photosynthesis, the pH and concentration of dissolved oxygen are subject to the greatest daily fluctuations. Some plants significantly affect the cationic composition of water and overall mineralization. For example, water soldier and Canadian pondweed significantly reduce the concentrations of major cations in environmental water (Brammer & Wetzel, 1984; Mulder et al., 2009; Martemyanov, 2014). On the contrary, the release of cations was observed during the decomposition of plants (Kudryavtsev & Kudryavtseva, 1982). As a rule, changes in the hydrochemical parameters of the environment induced by plants are within the tolerance of zooplankton species developing in the water bodies. Most of zooplankton species are habitat generalists. However, some factors may affect the intensity of reproduction and, consequently, the abundance of some species. For example, it is known that *Chydorus sphaericus* (O. F. Müller) has two development optima: at pH 5 and pH 9 (Manuilova, 1964). In our experiments, *Chydorus* reached the maximum number in the beds of submerged plants, where the environment was leached when saturated with oxygen in the daytime.

The results of some studies suggest that aquatic plants can secrete secondary metabolites that act as repellents on zooplankton or inhibit the development of individual species (Lauridsen & Lodge, 1996; Burks et al., 2000). However, many studies that indicate a high abundance and diversity of zooplankton in plant beds, as well as experiments that did not show an overwhelming effect on zooplankton of water with secretions of aquatic plants (Cerbin et al., 2007; Kurbatova et al., 2014) show that this factor does not become crucial in the formation of zooplankton in overgrown areas of water bodies. Apparently, the allelopathic or repellent effect of aquatic plants on planktonic crustaceans is manifested under certain conditions that have not been sufficiently studied so far. The type of hydrophyte, its development phase, density of beds, presence of competitors, pests, or other parameters may be important.

A higher species diversity of zooplankton was observed in the mixed phytocenosis than in the monospecies phytocenoses of the same plants. The combination of plants with different morphology and physiological activity creates a greater variety of ecological niches for zooplankton organisms. The share of predatory forms grew the most. The number of some species varied in proportion to the density of plants beds that stimulate their development (*Ceriodaphnia quadrangula* with water soldier, *Diaphanosoma brachyurum* with arrowhead), regardless of the presence of another species in the phytocenosis. In the mixed phytocenosis, the structure and quantitative parameters of zooplankton had their own characteris-

tics in comparison with zooplankton from the monospecies phytocenoses of the same plant species. In our experiment the abundance of *Daphnia longispina* became significantly lower in the mixed phytocenosis than in the control or in the treatment with a single species of plant. The overwhelming effect of water soldier on calanoid copepods and the stimulating effect of arrowhead on them were balanced in a mixed phytocenosis, and the abundance and biomass of these crustaceans remained close to the control.

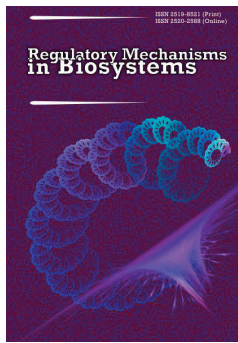
## Conclusion

Transformation of the aquatic environment as a result of the life activity of higher aquatic plants, as a rule, leads to an increase in the abundance and diversity of zooplankton. Increasing the heterogeneity of space and enriching the food supply due to the formation of phytogenic detritus and the lifetime release by plants of easily digestible organic substances are the main factors contributing to the growth of the abundance and number of zooplankton species. The species composition of phytocenoses and the belonging of plants to a particular ecological group are important for the formation of a zooplankton community in plant beds. The composition of the plant bed determines the hydrochemical parameters of the environment through the physiological characteristics of each individual species. The ecological type of plants determines the structure of space. The species similarity of zooplankton is closer between plants of the same ecological group than different ones. The relative number of predators in the community increases in the beds of plants. Not only phytophilic, littoral and small planktonic forms of zooplankton, but also relatively large euplanktonic ones can exist in the plant beds when sufficient food supplies exist and invertebrate predators are absent. The structure and quantitative characteristics of zooplankton have their own characteristics in monospecies and mixed phytocenoses of the same plant species. The results obtained are important for understanding the formation of zooplankton communities in the littoral of water bodies, the degree of their diversity, production potential, and functioning of aquatic ecosystems in general.

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## Effects of antihypertensive treatment on systemic inflammation, oxidative stress and proinflammatory cytokine levels

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Hypertension in its origin is a heterogeneous and multisystemic disease. Evaluation of oxidative stress activity based on the level of 8-iso-PgF<sub>2</sub>α, proinflammatory activity based on tumour necrosis factor-α, its type I soluble receptor, and C-reactive protein levels is relevant for further understanding of pathogenesis of hypertension and improvement of the early diagnostics of heart failure. 186 hypertensive patients were observed during a 2-months course of treatment, aged 30 to 65 years. Serum levels of 8-iso-PgF<sub>2</sub>α (n = 34), tumour necrosis factor-α and its type I soluble receptor were determined by ELISA before and after course of treatment. C-reactive protein level was determined by biochemical method. The control group included 16 clinically healthy individuals, aged 27 to 55 years. Hypertensive patients enrolled into the study were randomized into three groups that received different protocols of combined anti-hypertensive therapy: I clinical group – a combination of bisoprolol and indapamid, II – a combination of lacidipine and candesartan, III – a combination of fosinopril sodium and hydrochlorothiazide. On the background of combined antihypertensive therapy, we observed favourable dynamics of 8-iso-PgF<sub>2</sub>α, tumour necrosis factor-α and its type I soluble receptor, and C-reactive protein levels. Taking into account the insignificance of the correlations revealed, a one-factor dispersion analysis was applied which allowed us to determine the influence of the grade and duration of hypertension on the dynamics of the studied parameters. It has been found that the grade of hypertension is related to an increase in TNF-α and 8-iso-PgF<sub>2</sub>α serum levels, but not in TNF-α type I soluble receptor, and the duration of hypertension is related to an increase in C-reactive protein, TNF-α and its type I soluble receptor levels, with no relation to the level of 8-iso-PgF<sub>2</sub>α. Thus, oxidative stress possibly promotes the activation of potentially damaging immune mechanisms mediated by proinflammatory cytokines, nonspecific inflammation and drives the further progression of lesions in the target organs.

**Keywords:** 8-iso-PgF<sub>2</sub>α; tumour necrosis factor-α; type I soluble receptor; C-reactive protein; blood pressure.

### Introduction

Hypertension occupies one of the leading places in the structure of cardiac pathology and presents a complex medical and social problem considering its high prevalence and the early development of complications. Hypertension in its origin is a heterogeneous and multisystemic disease. In the last decade, the role of immune inflammatory activation mediated by proinflammatory cytokines, systemic inflammation and oxidative stress (OS) in the pathogenesis of cardiovascular diseases, including hypertension, has been actively studied (Bautista et al., 2005; Mahmud & Feel, 2005). The term “oxidative stress” (OS) is understood as a condition in which the amount of free radicals formed in the body is significantly higher than the activity of endogenous antioxidant systems that ensure their elimination (Kovalyova et al., 2005; Allison, 2016). It has been suggested that OS and immune inflammatory changes participate in the pathogenesis of cardiovascular dysfunction, are interrelated and can induce each other, forming a vicious circle (Kovalyova et al., 2015).

Among the proinflammatory cytokines, tumour necrosis factor-α (TNF-α) deserves special attention in the context of hypertension. Firstly, this is due to the fact that, as shown by experiments and an insignificant number of clinical studies, hemodynamic stress caused by increased blood pressure (BP) is one of the stimuli to increase production and release of pro-inflammatory cytokines, including TNF-α, into the bloodstream (Goldhaber et al., 1996; Grainger, 2007). Secondly, it is due to existing data on the ability of this cytokine to modulate the structure and function of the cardiovascular system through a number of mechanisms. As an example,

TNF-α is able to suppress myocardial contractility. This may be due to blocking of β-adrenergic signals, reduction of the content of nitric oxide in the heart, or changes in intracellular calcium homeostasis (Goldhaber et al., 1996). TNF-α can also induce structural changes in the myocardium of patients with hypertension and chronic heart failure, such as cardiomyocyte hypertrophy and interstitial fibrosis (Kovalyova & Ashcheulova, 2003). In addition, TNF-α promotes apoptosis of cardiomyocytes, and also activates metalloproteinases and depresses the expression of their inhibitors, thus promoting cardiac remodeling (Li et al., 2000; Haider et al., 2002) and eventually leading to cardiac dysfunction.

In healthy people, TNF-α is barely detectable in serum. Its level increases in infections under the influence of bacterial endotoxins (Vasan, 2006). There are two types of active receptors for TNF-α on the surface of almost all nuclear cell types that can virtually be cytokine targets. Soluble forms of receptors, which are considered endogenous inhibitors of TNF-α, are formed by separating the extracellular fragments of active receptors (Simbirsev, 2013). TNF-α type I receptor (sTNF-α RI) is the main mediator of cytokines' biological activity.

C-reactive protein (CRP) is a recognized marker of the acute phase of inflammation. With the advent of new highly sensitive techniques for its quantitative determination, it attracts increasing attention of cardiologists. This is due to the data on elevated CRP levels having a possible predictive role for the development of the set of cardio- and cerebrovascular complications such as congestive heart failure, myocardial infarction, stroke, sudden cardiac death and peripheral vascular disease (Sproston & Ashworth, 2018). The prognostic value of a high CRP level in patients with stable

angina pectoris, acute coronary syndrome and myocardial infarction has been shown (Melnikov et al., 2019; Melnikov et al., 2020). However, it should be noted that there were fewer reports on the diagnostic value of this systemic inflammation marker in essential hypertension, and there is insufficient evidence of the relationship between CRP, TNF- $\alpha$  and 8-iso-PgF $2\alpha$  (8-isoprostane) as the main OS marker in patients with hypertension from the clinical studies.

According to current data, detection of 8-isoprostane in blood or urine is a sensitive method for evaluating the intensity of OS. 8-iso-PgF $2\alpha$  is one of the most reliable and specific markers that allows us to assess the level of free radical production in the human organism in a wide spectrum of different diseases. 8-Isoprostane is a product of metabolism in reactions of arachidonic acid peroxidation that is isomeric to prostaglandin F $2\alpha$ . Its level is proportional to the amount of free radicals formed. This substance belongs to the family of eicosanoids that are a product of the non-enzymatic (free radical) oxidation of phospholipids of cellular biomembranes (Lawson et al., 1999; Cracowski et al., 2000). There is evidence (Greco et al., 2000; Czerska et al., 2016) of an increase in the level of 8-isoprostane in neurodegenerative diseases, coronary heart disease and hypertension. A significant increase in the level of 8-isoprostane has been observed in a number of conditions characterized by increased oxidative activity, particularly in tobacco smoking (Morrow et al., 1995), diabetes mellitus (Davi et al., 1999), hypercholesterolemia (Davi et al., 1997). Evaluation of OS activity based on the levels of 8-isoprostane, TNF- $\alpha$  and its soluble type I receptor, and C-reactive protein levels might allow us to reveal the correlations between the level of OS, immune activation and nonspecific inflammation in the human organism, which is relevant for further understanding of pathogenesis of hypertension and improvement of the early diagnostics of heart failure.

The purpose of this research was to assess the activity of proinflammatory cytokines and C-reactive protein serum levels (as independent markers of systemic inflammation) in the context of oxidative stress development, depending on the severity and duration of hypertension, and their changes under the influence of combined antihypertensive therapy.

## Materials and methods

The study was conducted in accordance with the current ethical requirements. The protocol of the study was approved by the Committee of Bioethics of Kharkiv National Medical University, Department of Propeutics of Internal Medicine No. 2 and Nursing Care. Informed consent was obtained from all participants of the study. 202 subjects were examined in the in-hospital setting, including 186 patients with essential hypertension of grades 1 to 3 who had asymptomatic lesions in the target organs (left ventricular hypertrophy/generalized retinal angiopathy/carotid intima-media thickening or presence of atherosclerotic plaques/microalbuminuria), aged 30 to 65 (mean 54.7  $\pm$  5.8) years, who previously had not been receiving regular antihypertensive therapy. The control group included 16 clinically healthy individuals (8 male and 8 female), aged 27 to 55 (mean 43.7  $\pm$  4.2) years, without cardiovascular, renal and endocrine anamnesis. Study participants included 149 female and 37 male patients, with the mean duration of disease of 10 years.

The Clinical Practice Guidelines for the Management of Arterial Hypertension of the European Society of Cardiology (ESC) / European Society of Hypertension (ESH) (2018) were used for verification of the diagnosis and assessment of the grade of hypertension. Exclusion criteria were: secondary hypertension, associated inflammatory and endocrine disorders (including diabetes mellitus) as well as other conditions that could have an impact on the activity of oxidative processes, including smoking anamnesis. Patients receiving statin therapy were not included into the study; statin-naïve patients who had indications for lipid-lowering therapy had been prescribed such after completion of the 2-month course of treatment. Of the patients enrolled into study, 33 had been diagnosed with essential hypertension of grade 1, 58 – of grade 2, and 95 – of grade 3. The level of 8-isoprostane as a main marker of OS was assessed in 34 patients, including 27 females and 7 males. Of this sub-group, 8 patients were diagnosed grade 1 hypertension, 8 – of grade 2, and 18 – of grade 3.

The contents of serum 8-isoprostane, TNF- $\alpha$  and its type I soluble receptor (sTNF- $\alpha$ RI) were determined in all subjects using the 8-isopro-

stane ELISA (“US Biological”, USA), ProCon TNF $\alpha$  (“Protein contour”, Russian Federation) and sTNF- $\alpha$ RI EASIA (“BioSource Europe S.A.”, Belgium) ELISA kits, respectively. The serum levels of C-reactive protein (CRP) were determined using the test-system for quantification of CRP (“Ukrmedservice Ltd.”, Ukraine).

Hypertensive patients enrolled into study had been randomized into three groups that received different protocols of combined anti-hypertensive therapy. The aforementioned parameters were reassessed after two months of treatment. I clinical group (n = 102) received a combination of a  $\beta$ -adrenoblocker (BAB) and diuretic (D) (bisoprolol 2.5–10.0 mg/day, and indapamid 1.5–2.5 mg/day). The daily dose of bisoprolol was administered by continuous slow titration, starting with low doses of 1.25 mg/day. Gradually the dose was increased to the maximum tolerated or target dose under the control of clinical parameters, especially blood pressure and heart rate (HR). II clinical group (n = 30) received a combination of a calcium channel blocker (CCB) and angiotensin receptor blocker (ARB) (lacidipine 2, 4 mg and candesartan 4, 8 and 16 mg). III clinical group (n = 54) received a combination of an angiotensin-converting enzyme inhibitor (ACEi) and diuretic (D) (fosinopril sodium 20 mg/day and hydrochlorothiazide 12.5 mg/day). The levels of 8-isoprostane were assessed in 10, 14, and 10 patients of the three clinical groups, respectively. The dose of medications was individually up-titrated in cases of need during the course of treatment. As one can see (Table 1) the different clinical groups were comparable in terms of age, gender structure and clinical course of hypertension.

**Table 1**  
Comparative characteristic of clinical groups of patients with hypertension

Parameters	I clinical group (n = 102)	II clinical group (n = 30)	III clinical group (n = 54)
Sex			
Male, n	20	6	11
Female, n	82	24	43
Age, years	54.5 $\pm$ 5.4	54.7 $\pm$ 5.8	54.5 $\pm$ 5.4
Hypertension history, years	9.6 $\pm$ 0.7	10.7 $\pm$ 1.4	9.7 $\pm$ 1.4
Hypertension grade, %			
1	17.6	23.3	14.8
2	28.4	26.7	38.8
3	54.0	50.0	46.4
New York Heart Association class, %			
0	11.9	16.7	16.6
I	19.6	20	22.2
II	65.6	60	59.3
III	2.9	3.3	1.9

The results were statistically processed using Statistica 7.0 software packages (StatSoft Inc., USA). Mean values and their standard deviation ( $\bar{x}$   $\pm$  SD) are presented in the text and tables. The intergroup analysis was performed using one-way analysis of variance (ANOVA) with the calculation of Fisher test (F); Wilcoxon T-test was used for paired intragroup analysis, with differences considered significant at  $P < 0.05$ .

## Results

Analysis of the activity of proinflammatory cytokines showed a significant increase in the levels of TNF- $\alpha$  in patients with hypertension compared to control: 187  $\pm$  18.1 vs 13.2  $\pm$  3.4 pg/mL, respectively ( $P < 0.001$ ). A similar tendency was observed with respect to soluble fraction of TNF- $\alpha$  receptor. The value of sTNF- $\alpha$ RI in hypertension also exceeded that in the control group: 2.14  $\pm$  0.28 vs 1.20  $\pm$  0.60 ng/mL, respectively ( $P < 0.001$ ). An increase in the serum 8-isoprostane was also found in patients with hypertension compared to healthy controls: 17.2  $\pm$  3.1 vs 1.41  $\pm$  0.25 pg/mL, respectively ( $P < 0.05$ ). The level of 8-isoprostane in hypertensive patients was 12.2 times higher compared to the control group. Considering the available data on the importance of CRP as a marker of systemic inflammation in cardiovascular pathology and as a predictor of development of heart failure (HF) and hypertension, we assessed the levels of serum CRP in patients with hypertension and revealed values exceeding the control group (6.23  $\pm$  0.33 mg/L). In order to elucidate the influence of not only the presence, but also the grade of increase in blood

pressure on the expression of TNF- $\alpha$ , sTNF- $\alpha$ RI, CRP, and also to evaluate the presence and level of oxidative stress, all patients were divided into groups depending on the grade of hypertension (Table 2).

Unlike TNF- $\alpha$  and 8-isoprostane, CRP levels did not significantly differ between groups. As one can see from Table 2, in patients with hypertension, a 3.2-fold, 7.1-fold and 18.4-fold increase (grades 1, 2, and 3, respectively) in the 8-isoprostane serum levels was observed compared to the control group. When comparing the levels of 8-iso-PGF $_{2\alpha}$  in patients with different grades hypertension, it was revealed that serum 8-isoprostane increased as the disease progressed: in patients with grade 3 hypertension, it exceeded by 5.8 times the value in patients with grade 1 and 2.6 times – in those with grade 2.

The levels of TNF- $\alpha$ , sTNF- $\alpha$ RI, 8-isoprostane, and CRP in the examined patients depended not only on the grade, but also on the duration of hypertension (Table 3).

Rank correlation analysis was performed to determine the relationship between blood pressure and levels of TNF- $\alpha$ , CRP and 8-isoprostane. The correlation coefficient ( $r$ ) between the grade of hypertension and serum TNF- $\alpha$  levels was 0.204 ( $P = 0.25$ ), for sTNF- $\alpha$ RI –  $r = -0.01$  ( $P = 0.97$ ), for 8-isoprostane –  $r = 0.11$  ( $P = 0.94$ ), for CRP –  $r = 0.02$  ( $P = 0.44$ ). Correlation coefficients with the duration of hypertension were: for TNF- $\alpha$  –  $r = 0.24$  ( $P = 0.17$ ), for sTNF- $\alpha$ RI –  $r = 0.17$  ( $P = 0.53$ ), for 8-iso-

prostane –  $r = 0.01$  ( $P = 0.95$ ), for CRP –  $r = 0.32$  ( $P = 0.74$ ). Taking into account the insignificance of the correlations revealed, a one-factor dispersion analysis was applied to determine the influence of the grade and duration of hypertension on the dynamics of the studied parameters levels. It was found that the grade of hypertension was related to the serum levels of TNF- $\alpha$  and 8-isoprostane ( $F = 9.58$ ,  $P = 0.002$  and  $F = 8.34$ ,  $P = 0.004$ , respectively), but not sTNF- $\alpha$ RI and CRP, and the duration of hypertension was related to the levels of TNF- $\alpha$ , sTNF- $\alpha$ RI and CRP ( $F = 6.72$ ,  $P = 0.003$ ;  $F = 2.34$ ,  $P = 0.006$ ;  $F = 9.96$ ,  $P = 0.002$ , respectively), with no relation to the level of 8-isoprostane. Analysis of the relationships between the levels of 8-isoprostane, TNF- $\alpha$  and sTNF- $\alpha$ RI has shown no significant correlations.

In the first group of patients on the background of treatment with bisoprolol and indapamide, the mean levels of TNF- $\alpha$  significantly decreased to 70.2 pg/mL compared to baseline before treatment, showing a 61.0% reduction (Table 4). The mean levels of soluble sTNF- $\alpha$ RI, which is a natural inhibitor of TNF- $\alpha$ , increased to 0.24 ng/mL (11.1%) after treatment. A significant decrease in the TNF- $\alpha$ /sTNF- $\alpha$ RI ratio by 34.4 (64.9%) indicated a predominant increase in the level of sTNF- $\alpha$ RI along with decrease in TNF- $\alpha$ . Since soluble forms of receptors act as natural antagonists of TNF- $\alpha$ , a decrease in this ratio reflects suppression of autoimmune and apoptotic activity in patients after treatment.

**Table 2**  
Levels of TNF- $\alpha$ , sTNF- $\alpha$ RI, CRP, and 8-isoprostane depending on the grade of hypertension ( $x \pm SD$ )

Parameter	Control group	Patients with hypertension		
		1 grade	2 grade	3 grade
TNF- $\alpha$ , pg/mL	13.2 $\pm$ 3.4	112.0 $\pm$ 26.1*	142.0 $\pm$ 27.2**	117.0 $\pm$ 18.5*
sTNF- $\alpha$ RI, ng/mL	1.20 $\pm$ 0.60	2.02 $\pm$ 0.22*	2.13 $\pm$ 0.16*	2.19 $\pm$ 0.08*
8-isoprostane, ng/mL	1.41 $\pm$ 0.25	4.48 $\pm$ 0.55***	10.00 $\pm$ 0.99****	25.90 $\pm$ 2.87****
CRP mg/L	6.23 $\pm$ 0.33	6.25 $\pm$ 0.57	6.01 $\pm$ 0.77	6.36 $\pm$ 0.45

Note: \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$  vs control group; \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$  vs patients with grade 1 hypertension; \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$  vs patients with grade 2 hypertension.

**Table 3**  
Levels of TNF- $\alpha$ , TNF- $\alpha$ RI, CRP, and 8-isoprostane in patients with different duration of hypertension, yrs ( $x \pm SD$ )

Parameter	Control group	Patients with duration of hypertension, yrs		
		<5	5–10	>10
TNF- $\alpha$ , pg/mL	13.2 $\pm$ 3.4	108.0 $\pm$ 23.0*	128.0 $\pm$ 25.8*	145.0 $\pm$ 28.4***
sTNF- $\alpha$ RI, ng/mL	1.20 $\pm$ 0.60	2.08 $\pm$ 0.21*	2.14 $\pm$ 0.18*	2.20 $\pm$ 0.19*
8-isoprostane, ng/mL	1.41 $\pm$ 0.25	9.17 $\pm$ 1.72***	19.30 $\pm$ 4.09****	14.90 $\pm$ 3.79***
CRP mg/L	6.23 $\pm$ 0.33	6.08 $\pm$ 0.65	6.28 $\pm$ 0.51	6.38 $\pm$ 0.59

Note: \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$ , vs control group; \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$  vs patients with duration of hypertension < 5 years; \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$  vs patients with duration of hypertension 5–10 years.

**Table 4**  
Dynamics of the cytokines, CRP and 8-isoprostane in the course of combined antihypertensive therapy in the observed patients ( $x \pm SD$ )

Parameter	I group	II group	III group
TNF- $\alpha$ , pg/mL, prior to treatment	115.0 $\pm$ 19.6	133.0 $\pm$ 22.6	78.2 $\pm$ 23.7
TNF- $\alpha$ , pg/mL, after 2 months treatment	44.8 $\pm$ 8.21***	45.0 $\pm$ 5.63***	40.6 $\pm$ 15.8***
sTNF- $\alpha$ RI, ng/mL, prior to treatment	2.17 $\pm$ 0.12	2.10 $\pm$ 0.16	2.25 $\pm$ 0.21
sTNF- $\alpha$ RI, ng/mL, after 2 months treatment	2.41 $\pm$ 0.03*	2.63 $\pm$ 0.53*	2.38 $\pm$ 0.19
TNF- $\alpha$ /sTNF- $\alpha$ RI, prior to treatment	53.0 $\pm$ 17.4	63.3 $\pm$ 19.3	34.8 $\pm$ 12.2
TNF- $\alpha$ /sTNF- $\alpha$ RI, after 2 months treatment	18.6 $\pm$ 7.8**	17.1 $\pm$ 6.4**	17.1 $\pm$ 6.6**
8-isoprostane, ng/mL, prior to treatment	20.5 $\pm$ 17.4	12.7 $\pm$ 9.6	20.2 $\pm$ 11.9
8-isoprostane, ng/mL, after 2 months treatment	12.3 $\pm$ 7.27*	2.4 $\pm$ 1.5*	10.2 $\pm$ 7.61*
CRP mg/L, prior to treatment	5.86 $\pm$ 0.48	6.81 $\pm$ 0.59	6.30 $\pm$ 0.49
CRP mg/L, after 2 months treatment	3.85 $\pm$ 0.36	3.68 $\pm$ 0.38*	5.14 $\pm$ 0.42

Note: \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$  vs levels prior to treatment.

In the second group of patients, on the background of treatment with a combination of lacidipine and candesartan, the mean TNF- $\alpha$  levels decreased by 88 pg/mL (66.2%) compared to the baseline before treatment (Table 4). With respect to sTNF- $\alpha$ RI, the reverse trend was observed, that is, an increase in its mean level by 0.53 ng/mL (25.2%) after treatment. A 73.0% decrease in TNF- $\alpha$ /sTNF- $\alpha$ RI ratio reflected a significant decrease in the level of autoimmune activation under the influence of 10-week ARB + CCB therapy. In the third group of patients, the combination of fosinopril sodium with hydrochlorothiazide significantly reduced the mean TNF- $\alpha$  levels by 37.6 pg/mL (48.1%). With respect to sTNF- $\alpha$ RI, an insignificant increase in its mean level after treatment by 0.13 ng/mL

(5.8%) was observed. The value of TNF- $\alpha$ /sTNF- $\alpha$ RI ratio decreased by 17.7 (by 50.9%), compared to baseline before treatment, which also reflected a decrease in the level of autoimmune activation under the influence of therapy.

Analyzing the obtained data with respect to the level of 8-isoprostane two months from the start of therapy on the background of treatment, in the third group patients, its level decreased by 50% (2-fold) from the initial level. In the second clinical group, the reduction in serum levels of 8-isoprostane by 80.9% (5.2-fold) was observed after 2 months of treatment compared with the baseline. In the first group of patients, the levels of 8-isoprostane also decreased by 40% (1.7-fold) from the initial level after

two months of therapy. Thus, different protocols of combined therapy with beta-blocker plus diuretic (bisoprolol and indapamide) and ACE inhibitor plus diuretic (fosinopril and hydrochlorothiazide) expressed a similar effect on the dynamics of 8-isoprostane levels during two months of treatment. Analyzing the dynamics of CRP on the background of combined treatment, we obtained the following data: the mean values of serum CRP had decreased by 2.01 mg/L (34.3%), by 3.13 mg/L (46.0%) by 1.16 mg/L (18.4%) in the I, II and III clinical groups, respectively. Under

the influence of combined antihypertensive therapy with all three schemes, there was an improvement in clinical status, expressed by a decrease in the intensity and frequency of headaches, dizziness, pain in the heart area, fatigue, and an increase in exercise tolerance as a result of significant reduction in blood pressure (Table 5). All patients who received treatment with one of the combined anti-hypertensive therapy regimens were discharged from the hospital (on the 10–14th day of treatment) in a satisfactory condition.

**Table 5**  
Blood pressure and heart rate dynamics during the course of treatment ( $x \pm SD$ )

Parameter	I group	II group	III group
Systolic blood pressure prior to treatment	168.3 ± 26.6	176.0 ± 27.2	180.2 ± 28.6
Systolic blood pressure after 2 weeks of treatment	129.5 ± 11.9***	131.6 ± 11.0***	132.6 ± 9.7***
Diastolic blood pressure prior to treatment	100.8 ± 12.7	105.2 ± 14.1	107.1 ± 11.3
Diastolic blood pressure after 2 weeks of treatment	75.5 ± 6.3***	75.3 ± 5.0***	76.5 ± 5.8***
Average BP blood pressure prior to treatment	123.3 ± 16.0	128.8 ± 17.6	131.3 ± 15.7
Average BP blood pressure after 2 weeks of treatment	93.4 ± 6.5***	93.9 ± 6.2***	95.0 ± 5.7***
Pulse blood pressure prior to treatment	67.5 ± 19.9	71.2 ± 18.0	73.1 ± 22.2
Pulse blood pressure after 2 weeks of treatment	54.0 ± 11.8***	55.9 ± 10.6***	55.6 ± 10.1***
HR prior to treatment	83.4 ± 11.9	74.2 ± 9.2	78.4 ± 12.1
HR after 2 weeks of treatment	75.3 ± 6.2*	76.7 ± 4.8	73.1 ± 6.6*

Note: \* –  $P < 0.05$ ; \*\* –  $P < 0.01$ ; \*\*\* –  $P < 0.001$ ; vs levels prior to treatment.

In patients treated with bisoprolol and indapamide, a reduction in the mean values of office systolic blood pressure (SBP) by 38.8 mm Hg, office diastolic blood pressure (DBP) by 25.3 mm Hg, average BP by 29.8 mm Hg, pulse BP by 13.5 mm Hg during the period of inpatient treatment was observed (23.1%, 25.1%, 24.2%, and 20.0%, respectively vs levels prior to treatment, Table 5). In the group of patients who received candesartan and lacidipine, there were similar changes during the inpatient treatment in the mean values of the office SBP by 44.5 mm Hg, office DBP 29.9 mm Hg, mean BP by 34.8 mm Hg, pulse BP by 15.2 mm Hg (25.3%, 28.4%, 27.1% and 21.4%, respectively vs levels prior to treatment). In the third group (patients treated by fosinopril sodium and hydrochlorothiazide), during the period of inpatient treatment, there was a decrease in the average values of office SBP by 47.6 mm Hg, office DBP by 30.6 mm Hg, mean BP by 36.3 mm Hg, pulse BP by 17.5 mm Hg (26.4%, 28.6%, 27.6% and 24.0%, respectively vs levels prior to treatment). Thus, there was a significant decrease in both office SBP and DBP and, accordingly, mean BP and pulse BP in enrolled patients, regardless of the group of drugs they received. The level of blood pressure reduction allowed us to reach the target values for both SBP and DBP.

A change was also found in the average heart rate during the inpatient treatment period: a decrease in the heart rate in patients taking bisoprolol plus indapamide and fosinopril plus hydrochlorothiazide by 8 bpm (9.6%) and by 5 bpm (6.7%), respectively. Conversely, in patients of group II HR tended to increase by 3 bpm (3.4%) on average.

## Discussion

The mean value of TNF- $\alpha$  was 14.2 times higher compared to control values, suggesting the possible role of hemodynamic stress as one of the stimuli for increasing synthesis and release of pro-inflammatory cytokines, in particular TNF- $\alpha$ , into the circulation (Grander, 2004; Azra & Feel, 2005; Bautista et al., 2005). The mean value of sTNF- $\alpha$ RI in patients with increased TNF- $\alpha$  activity (likely, due to increased blood pressure) was 1.78 times, or 78.3% higher, compared to normal subjects.

Numerous epidemiological studies have shown a relationship between tachycardia and hypertension. Increased heart rate is associated with many risk factors, including dyslipidemia, hyperinsulinemia, obesity, and elevated hematocrit. Tachycardia is a marker of increased activity of the sympathetic nervous system. It is known that a decrease in heart rate correlates with longer life in mammals. Moreover,  $\beta$ -blockers affect the renin-angiotensin-aldosterone system (RAAS), inhibiting the release of renin by blockade of  $\beta$ 1-receptors in juxtaglomerular apparatus of the kidneys. Bisoprolol is a  $\beta$ -blocker with a high degree of cardioselectivity (1:75); it does not affect the level of atherogenic fractions of lipids (triglycerides, cholesterol, low-density lipoproteins) in prolonged administration (Ostroumova & Maksimov, 2012; Knjaz'kova, 2013). The combination of

bisoprolol with indapamid is able to improve endothelial function due to the presence of antioxidant properties. An important role in this combination is played by indapamid, which, unlike other diuretics, has a direct vasodilator action (London, 2004).

Lacidipine occupies a special place among the CCBs, showing the most pronounced anti-atherosclerotic properties. Its antioxidant activity is comparable to that of vitamin E and exceeds that of all other CCB (Svishchenko, 2002). Lacidipine forms a stronger bond to the cellular membrane compared to other CCBs. This determines its high local concentration in the tissues of blood vessels (to which it is highly selective), which is particularly important in conditions of hypercholesterolemia, when the cellular membrane's availability to CCBs is decreased due to high cholesterol load. Lacidipine's binding to the cellular membrane remains strong even in the described conditions, being significantly higher than with other CCBs (Herbette, 1995). The tight bond with the membrane defines a longer duration of lacidipine action and its ability to intervene in the majority of intracellular processes at the early stages of atherogenesis. The main role in the action of the drug on various links of atherosclerotic process is given to its high vascular selectivity and the ability to tightly bind to the cellular membrane in conjunction with an intense antioxidant effect and the ability to optimize the calcium homeostasis. Importantly, the anti-atherogenic effect of lacidipine is not determined by its antihypertensive effect: it is observed even when using lacidipine in low doses that do not affect blood pressure (Svishchenko, 2002).

Fosinopril is the only angiotensin-converting enzyme inhibitor (ACEi) that retains the phosphinic acid residue in its chemical formula; it prevents conversion of angiotensin I to angiotensin II, resulting in reduction of vasopressor activity and aldosterone secretion (Preobrazhenskij & Savchenko, 2000; Dzijak et al., 2005). Fosinopril is de-esterified in the liver or gastrointestinal mucosa and is converted to its active form, fosinoprilate (Ageev & Mareev, 2000; Sirenko & Rekovec, 2005). Fosinoprilate competitively binds to ACE, preventing ACE from binding to and converting angiotensin I to angiotensin II. Inhibiting the production of AII lowers peripheral vascular resistance, decreases afterload, and decreases blood pressure, thus helping to alleviate the negative effects of AII on cardiac performance. The second mechanism of action of ACEi on endothelial function is their ability to prevent degradation of bradykinin (Greene, 2000), as ACE is identical to the kinase II enzyme, which causes the conversion of bradykinin to its inactive form. Bradykinin is a potent stimulator of the release of endothelium-dependent relaxing factors such as nitric oxide, endothelium hyperpolarization factor and prostacyclin (PGI<sub>2</sub>).

The advantage of fosinopril is the balanced double way of excretion – slightly more than half of the total amount of the drug (54.0%) is excreted by the kidneys with urine, the rest (46.0%) – by hepatic degradation of active metabolites with their sequential release through the gastrointestinal tract. It is important to note that the decrease in renal filtration increases

proportionally the hepatic route of excretion of the drug, and on the contrary, in liver disease – increases the contribution of renal excretion. Actually, this pharmacokinetic feature of fosinopril is the basis of the important clinical recommendations: no additional correction of fosinopril doses is usually required in renal and hepatic failure (Ageev & Mareev, 2000; Karpov, 2001). This characteristic feature of fosinopril makes it a safer choice than other ACEi for heart failure patients with impaired kidney function resulting from impaired renal perfusion as fosinopril can still be eliminated by the liver, preventing excessive increase of its serum levels.

The results of current study allow us to suggest that in individuals with grade 1 hypertension, increase of sTNF- $\alpha$ RI ( $2.02 \pm 0.22$  ng/mL) at high levels of the circulating TNF- $\alpha$  ( $112 \pm 26$  ng/mL) can possibly be regarded as an adaptive mechanism that, on one hand, reduces the number of active receptors on the surface of target cells, and on the other hand, may neutralize the bioactivity of TNF- $\alpha$  through the soluble receptor forms (sTNF- $\alpha$ RI) binding with the latter. In patients with grade 2 hypertension, a parallel increase in sTNF- $\alpha$ RI ( $2.13 \pm 0.16$  ng/mL) was probably not enough to neutralize the negative effect of TNF- $\alpha$  ( $142 \pm 27$  ng/mL), as could be evidenced by a further increase in the TNF- $\alpha$ /sTNF- $\alpha$ RI ratio (66.7 vs 55.4 in grade 1 hypertension and 11.0 in normotensive individuals). At the maximal levels of blood pressure the levels of TNF- $\alpha$  were decreasing ( $117 \pm 19$  ng/mL), which possibly could be due to its binding to sTNF- $\alpha$ RI. On the other hand, we can suggest the possibility of TNF- $\alpha$  triggering a cascade leading to apoptosis of cells producing cytokine. The further increase in sTNF- $\alpha$ RI on the background of a decrease in TNF- $\alpha$  has a positive significance. The fact that the TNF- $\alpha$ /sTNF- $\alpha$ RI value was 53.4, with the TNF- $\alpha$  levels also still exceeding those in grade 1 hypertension and healthy individuals, could indicate that such a level of sTNF- $\alpha$ RI was unable to inactivate TNF- $\alpha$  cytotoxicity.

According to the single-factor dispersion analysis, there was an increase in the CRP serum levels in patients with hypertension. This increase depended only on the duration of hypertension, but not on the grade of BP elevation; there was also no significant correlation between CRP and BP, possibly being consistent with existing data on the definition of CRP as an independent risk factor for hypertension. In particular, an increased level of CRP had been shown in normotensive individuals who later developed essential hypertension. The increased level of CRP is related to the deterioration of endothelium-dependent relaxation, posing a potential risk of developing hypertension. In the hypertensive population, it is established that CRP is an independent predictor of atherosclerosis progression, more significant than pulse and systolic blood pressure. Our findings suggest that elevated CRP levels can be considered as a marker of systemic inflammation in hypertensive patients (Bautista, 2001; Onat et al., 2008).

Taking into account the correlations revealed, it can indicate the role of OS in the pathogenesis of hypertension as a damaging mechanism that promotes the activation of immune mechanisms, nonspecific inflammation and the further progression of the disease. OS causes damage to the vascular endothelium, and the main disorders in the vascular wall that are characteristic for hypertension are endothelial dysfunction and hypertrophy of the smooth muscle cells.

## Conclusions

The obtained data allows us to suggest the possible role of oxidative stress as one of the important pathogenetic mechanisms in hypertension, manifested in this study by an increase in serum 8-isoprostane levels compared to clinically healthy individuals. Thus, oxidative stress possibly promotes the activation of potentially damaging immune mechanisms mediated by pro-inflammatory cytokines, nonspecific inflammation and drives the further progression of lesions in the target organs.

An increase in circulating TNF- $\alpha$ , sTNF- $\alpha$ RI, CRP and 8-isoprostane levels supports data on involvement of immune activation and oxidative stress in pathogenesis of hypertension. It was found that the grade of hypertension influences the increase in the serum levels of TNF- $\alpha$  and 8-iso-PgF $2\alpha$ , but not of sTNF- $\alpha$ RI, and the duration of hypertension affects the activity of the CRP, TNF- $\alpha$  and sTNF- $\alpha$ RI, with no effect on the levels of 8-iso-PgF $2\alpha$ .

The data obtained in the course of two months treatment testifies to the anti-inflammatory and anti-apoptotic effects of the combined antihypertensive therapy. The most pronounced result was observed in individuals receiving combined therapy with lacidipine plus candesartan and bisoprolol plus indapamide.

It is important that further studies are made to assess the activity of proinflammatory cytokines, namely tumour necrosis factor- $\alpha$  and its type I soluble receptor, levels of C-reactive protein as an independent marker of systemic inflammation, levels of 8-isoprostane as a marker of oxidative stress, involving larger study populations with long follow-up periods. The results of advanced research will help in the formation of pathogenetically justified and prognostically significant antihypertensive therapy regimens.

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