O ESTADO DA MEMBRANA DE CÉLULAS VERMELHAS EM ANEMIA DE DEFICIÊNCIA DE FERRO EM MULHERES DE IDADE REPRODUTIVA**THE STATE OF THE RED BLOOD CELL MEMBRANE IN IRON DEFICIENCY ANAEMIA IN WOMEN OF REPRODUCTIVE AGE****СОСТОЯНИЕ ЭРИТРОЦИТАРНОЙ МЕМБРАНЫ ПРИ ЖЕЛЕЗОДЕФИЦИТНОЙ АНЕМИИ У ЖЕНЩИН ФЕРТИЛЬНОГО ВОЗРАСТА**

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RESUMO

O problema da deficiência de ferro é urgente em muitos países, independentemente dos padrões de vida socioeconômicos. Entre os problemas biomédicos, o estudo dos parâmetros bioquímicos do sangue é de suma importância. Um grande interesse em vários aspectos dessa questão não é acidental, já que a função de transporte do sangue depende amplamente dela e, conseqüentemente, da entrega eficiente de oxigênio, glicose, aminoácidos, gorduras, vitaminas, sais minerais, hormônios, mediadores, anticorpos e metabolitos para as microzonas nos tecidos. O objetivo deste artigo é estudar o estado hematológico e o estado da membrana eritrocitária em mulheres em idade reprodutiva que sofrem de anemia ferropriva (ADF). A anemia por deficiência de ferro (ADF) é uma combinação de sintomas clínicos e hematológicos, caracterizada pela formação deficiente de hemoglobina devido à deficiência de ferro no soro e medula óssea, bem como o desenvolvimento de distúrbios tróficos nos órgãos e tecidos. Durante o estudo, um hemograma completo foi realizado utilizando um ensaio imunoenzimático, utilizando os kits produzidos pela JSC Vector-Best, e o método de Kamyshnikov. Com base nos resultados do estudo da capacidade de sorção de eritrócitos (ESC) em mulheres em idade reprodutiva com ADF, estabeleceu-se que este parâmetro é 1,2 vezes maior do que no grupo controle. O desenvolvimento de anemia está associado a mudanças significativas na estabilidade osmótica dos eritrócitos, o que resulta em um aumento na proporção de eritrócitos instáveis e de alta resistência. A novidade científica do artigo é que os autores estabeleceram os parâmetros-chave para detecção de anemia por deficiência de ferro.

Palavras-chave: *deficiência de ferro, sistema sanguíneo, teor de hemoglobina, membrana eritrocitária, resistência osmótica.*

ABSTRACT

The problem of iron deficiency is urgent in many countries, regardless of the socio-economic standards of living. Among biomedical problems, the study of biochemical blood parameters is of paramount importance. A great interest in various aspects of this issue is not accidental, since the transport function of the blood largely depends on it and, consequently, the efficient delivery of oxygen, glucose, amino acids, fats, vitamins, mineral salts, hormones, mediators, antibodies and metabolites to the micro-zones in the tissues. The purpose of this article is to study the hematological status and the state of the red blood cell membrane in women of reproductive age suffering from iron deficiency anemia (IDA). Iron deficiency anemia (IDA) is a combination of clinical and hematological symptoms characterized by impaired hemoglobin formation due to iron deficiency in serum and bone marrow, as well as the development of trophic disorders in the organs and tissues. During the study, a complete blood count was performed using an enzyme-linked immunosorbent assay, using the kits produced by JSC Vector-Best, and the Kamyshnikov method. Based on the results of the study of the erythrocyte sorption capacity (ESC) in women of reproductive age with IDA, it was established that this parameter is 1.2 times higher than that in the control group. The development of anemia is associated with significant changes in the osmotic stability of the erythrocytes, which results in an increase in the proportion of both unstable and high-resistance erythrocytes. The scientific novelty of the article is that the authors established the key parameters for iron deficiency anemia detection.

Keywords: *iron deficiency, blood system, hemoglobin content, erythrocyte membrane, osmotic resistance.*

АННОТАЦИЯ

Проблема железодефицитных состояний актуальна в многих странах, независимо от социально-экономического уровня жизни. Среди биомедицинских проблем изучение биохимических показателей крови имеет первостепенное значение. Большой интерес к различным аспектам этой проблемы не случаен, так как от нее во многом зависит транспортная функция крови и, следовательно, эффективный транспорт кислорода, глюкозы, аминокислот, жиров, витаминов, минеральных солей, гормонов, медиаторов, антител и метаболитов к микрозомам в тканях. Цель работы – изучить гематологический статус и состояние мембран эритроцитов женщин фертильного возраста, страдающих ЖДА. Железодефицитная анемия (ЖДА) представляет собой сочетание клинических и гематологических симптомов, характеризующихся нарушением образования гемоглобина из-за дефицита железа в сыворотке и костном мозге, а также развитием трофических нарушений в органах и тканях. В ходе исследования проводился общий анализ крови, использовался метод твердофазного иммуноферментного анализа, с использованием наборов производства ЗАО «Вектор-Бест» и метод Камышникова. По результатам изучения сорбционной емкости эритроцитов у женщин фертильного возраста с ЖДА установлено, что данный показатель повышен в сравнении с показателем контрольной группы в 1,2 раза. Развитие анемии связано со значительными изменениями осмотической стабильности эритроцитов, что приводит к увеличению доли как нестабильных, так и эритроцитов с высокой резистентностью. Научная новизна статьи в том, что авторы установили, какие показатели являются ключевыми при выявлении железодефицитной анемии.

Ключевые слова: *дефицит железа, система крови, содержание гемоглобина, эритроцитарная мембрана, осмотическая резистентность.*

1. INTRODUCTION

The problem of iron deficiency is urgent both in the countries with a low socio-economic level and in economically developed countries. According to the estimates of the World Health Organization (2002), iron deficiency (ID) of various severity affects about 4 billion people, representing more than 60% of the world's population. Of these, IDA accounts for almost 2 billion, which makes IDA the most common disease in the world and the most common type of anemia (90%) (WHO: The World Health Report, 2002).

Iron deficiency anemia (IDA) is a combination of clinical and hematological symptoms characterized by impaired hemoglobin formation due to iron deficiency in serum and bone marrow, as well as the development of trophic disorders in the organs and tissues. The incidence of IDA is highest in children 12-17 years old, pregnant women and women of reproductive age (Konovodova and Yakunina, 2010; Tikhomirov and Kocharyan, 2006). The highest ID rates are observed in these risk groups. Thus, ID is observed in more than 51% of women of childbearing age, which is associated with monthly blood loss and pregnancies (Malkoch *et al.*, 2013; Akkaya *et al.*, 2019; Gou *et al.*, 2019).

Changes in the blood, as the internal environment of the body, are the middle link between the fastest respiratory and circulatory reflex reactions and the slowest changes in metabolism (AlZahrani and Al-Sewaidan, 2017; Gallo *et al.*, 2017). The blood system has long been a reliable clinical indicator for the assessment of body condition. Due to its high reactivity, it acts as an effector in the implementation of adaptive and trophic influences of the sympathetic nervous system (Kotelnikova, 2000; Burkitbaiev *et al.*, 2017; Nobre *et al.*, 2018). Among biomedical problems, the study of biochemical blood parameters is of paramount importance.

A great interest in various aspects of this issue is not accidental, since the transport function of the blood largely depends on it and, consequently, the efficient delivery of oxygen, glucose, amino acids, fats, vitamins, mineral salts, hormones, mediators, antibodies and metabolites to the micro-zones in the tissues (Ciana *et al.*, 2017). The process of adaptation of the body to adverse environmental factors is accompanied by changes in metabolic processes in the tissues and cells, which play an important role in haemato-histo-lymphatic metabolism. This process depends on many factors, including the red blood cell membrane stability (Prokopenko, 1995; Tong

et al., 2018; Das *et al.*, 2018). Increased stability of the cell membranes maintains an optimal number of cells in the blood that is necessary for the physiological course of metabolic processes. The aim of the study was to investigate the hematological status and the state of the red blood cell membranes in women of reproductive age suffering from IDA.

2. MATERIALS AND METHODS

The study involved women of reproductive age with 1-2 stage IDA and healthy women aged 18-50 years. A total of 50 patients were examined (30 women with IDA, 20 women in the control group). The diagnosis and severity of IDA were verified in accordance with the recommendations of the International Classification of Diseases and the Haematology Manual (Vorobyeva, 1985; Chowdhury *et al.*, 2018; Klichkhanov and Dzharfarova, 2018). Complete blood count was performed on the MS4 hematological automatic analyzer (Meletschloesing Laboratories, France) with determination of hemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC) and red blood cell indices – mean cell hemoglobin (MCH), mean cell volume (MCV).

The following parameters were determined using the A-25 BioSystems biochemical semi-automatic analyzer (Spain), using diagnostic kits manufactured by JSC Vector-Best: serum iron levels (SI), total serum iron binding capacity (TSIBC), creatinine and urea. Serum ferritin levels were determined using the Alisei Q.S. ELISA automated analyzer (SEAC, Italy) by the method of enzyme-linked immunosorbent assay, using the kits manufactured by JSC Vector-Best.

The osmotic stability of the erythrocyte membrane was determined using the Kamyschnikov V.S. method (Kamyschnikov, 2003; Sawyer *et al.*, 2018; Eapen *et al.*, 2018). The method is based on the determination of the percentage of destroyed red blood cells in the urea solution of various concentrations: 33%, 38%, 44%, 50%, 55%, 61%, 100%. Venous blood was collected in the morning on an empty stomach into heparinized tubes. The erythrocytes (0.3 ml) were washed with cooled isotonic sodium chloride solution (0.6 ml). 100 μ L of washed red blood cells were added to each of 7 centrifuge tubes with 5.0 ml of urea solutions with different concentrations. After 10 minutes of incubation, the tubes were centrifuged for 10 minutes at 1500 rpm. The extinction above the sedimentary liquid was measured at λ 540 nm using a spectrophotometer. The percentage of haemolysed cells was

determined by the formula: 1. $E7 = 100\%$, 2. $E1-6 = x\%$ 3. $X = E1-6 \times 100\% / E7$. To study the sorption capacity, the erythrocytes were separated from the plasma and washed three times with chilled saline. The erythrocyte sorption capacity (ESC) was evaluated using the A.A. Togaybayev method modified by T.V. Kopytova (Kopytova, 2006; Sutil-Vega *et al.*, 2019; Minetti *et al.*, 2018). Units %.

3. RESULTS AND DISCUSSION:

The analysis of the hematological parameters of the subjects showed that mean hemoglobin levels (HGB) in healthy women were 132.1 ± 1.8 g/L, and 101.9 ± 4.3 g/L in women with IDA. Mean red blood cell (RBC) counts in the control group were 4.4 ± 0.08 and $4.1 \pm 0.1/10^6/\text{mL}$ in the IDA group. Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were lower in the IDA group compared to the control group and were 29.6 ± 0.2 and 22.8 ± 1.28 (p=0.00002) and 333.96 ± 7.76 and 315 ± 13.19 pg, respectively (p=0.385). Mean cell volume (MCV) was lower in IDA and was 73.2 ± 2.1 fL and 85.3 ± 0.9 fL in healthy women (p=0.004). The studied parameters are presented in Table 1.

Although serum iron (SI) and ferritin levels in women with IDA were within the normal range, they were significantly lower compared to the control group (10.1 ± 2.0 $\mu\text{mol/L}$, 31.21 ± 5 ; 3 ng/mL and 15.37 ± 1.1 $\mu\text{mol/L}$, 86.81 ± 5.05 ng/mL, respectively). Serum iron binding capacity in these women was increased and reached 73.61 ± 5.68 $\mu\text{mol/L}$, and in healthy women, it was 53.0 ± 2.6 $\mu\text{mol/L}$. The study findings are presented in Table 1.

Prolonged hypoxemia in chronic IDA results in changes in the protein-phospholipid layer of the erythrocyte membrane, activation of lipids and protein peroxidation, and the formation of a "hard" membrane. One of the indicators of changes in the properties of the cell membranes is its sorption capacity. The analysis of the erythrocyte sorption capacity (ESC) provides information on the erythrocyte recovery, which changes together with changes in the plasma barrier properties. Decreased ESC is interpreted as energy deficiency in the red blood cells. Elevated ESC is considered an indicator of membrane damage and cellular disorganization (Kopytova, 2006; Grzeskowiak *et al.*, 2018; Vinogradov *et al.*, 2017; Alshalani *et al.*, 2018). According to the results of the ESC studies in women of reproductive age with IDA, this parameter is increased compared to the control group (Table 2).

An increase in the ESC in patients with IDA can be considered as an indicator of membrane damage. The membrane damage may be caused by oxidative stress, which develops during hypoxia associated with iron deficiency. In addition to the sorption capacity, the lability of the erythrocyte membrane depends on such physiological properties as deformability, ability to aggregate and osmotic stability, ensuring the promotion of red blood cells in the bloodstream and, consequently, oxygen transport to the organs and tissues. The results of the study of the erythrocyte osmotic stability are presented in Table 3.

The data in Table 3 shows a significant change in the osmotic stability of the erythrocytes in women with anemia at various stages. In the group of patients with I stage anemia (group 1), a higher percentage of low-resistant erythrocytes was observed (hemolysis increased in low-percentage urea solutions), and the percentage of haemolysed cells in the solution, 33%, and 38%, was significantly different from the control group, i.e., 7- and 4.3-fold, respectively. At the same time, in solutions with a high concentration of the area, the percentage of haemolysed cells was significantly lower than in the control group, indicating an increase in the proportion of the osmotically stable erythrocytes in women with I stage anemia. The proportion of haemolysed cells in a 61% urea solution was significantly lower compared to the control group, i.e., by 43%.

In the group of patients with II stage anemia (group 2), the same tendency was observed but even more pronounced. Thus, in the solutions with a low concentration of the urea, the percentage of low-resistant erythrocytes was lower than in the first group but rather higher than in the control group: in 33% solution – 5.9 times, 38% and 50% solution – 3.25 times. At the same time, the percentage of highly resistant erythrocytes in 55% and 61% urea solutions was significantly lower compared to the control group, i.e., 3.2- and 2.8-fold, respectively, and compared to group 1, i.e., by 48% and 24%, respectively. In the patients of group 2, 12% more resistant cells were observed in 100% urea solution, compared to the controls. The results of the erythrocyte osmotic stability studies in the patients with I and II stage anemia are presented in the form of osmotic stability curves (Figure 1).

The osmotic stability curve for the control group has the form of a graph of a direct linear dependence: the percentage of haemolysed cells is directly proportional to the concentration of urea in the solution. The erythrocyte osmotic stability curve for the patients of group 1 (with I stage

anemia) is inversely related to the proportion of haemolysed cells in low and medium concentrated solutions of urea. In the patients of group 2, the erythrocyte osmotic stability curve has the same pattern as that in the patients of group 1, with a more significant tendency to a decrease in the degree of hemolysis in the urea solution in almost all studied concentrations.

The observed changes in the erythrocyte osmotic stability in patients with anemia of various severity can be interpreted as changes in the physicochemical properties of the erythrocyte membranes under conditions of increasing hypoxia. In hypoxia, the activation of lipid and oxidative protein metabolism in cell membranes causes their chemical and conformational rearrangement, which can lead to the appearance of cells that are not resistant to the osmotic pressure of their environment and create conditions for reduced membrane permeability, and thereby increase cell resistance to osmotically active substances (Kanuri *et al.*, 2018; Vazquez-Martin *et al.*, 2016; He *et al.*, 2017; Alshalani and Acker, 2017).

Therefore, the development of anemia is accompanied by significant changes in the osmotic stability of erythrocytes, which results in an increase in the proportion of both unstable erythrocytes and high-resistance erythrocytes. This is reflected in changes in the pattern of the erythrocyte osmotic stability curve. Exacerbation of anemia with the same pattern of osmotically resistant cell redistribution is associated with higher quantitative parameters of the observed changes.

4. CONCLUSIONS:

Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were lower in the IDA group compared to the control group and were 29.6 ± 0.2 and 22.8 ± 1.28 ($p=0.00002$) and 333.96 ± 7.76 and 315 ± 13.19 pg, respectively ($p=0.385$). Mean cell volume (MCV) was lower in IDA and was 73.2 ± 2.1 fL and 85.3 ± 0.9 fL in healthy women ($p=0.004$).

Serum iron (SI) and ferritin levels in women with IDA were within the normal range, it was significantly reduced compared to the control group (10.1 ± 2.0 $\mu\text{mol/L}$, 31.21 ± 5 ; 3 ng/mL and 15.37 ± 1.1 $\mu\text{mol/L}$, 86.81 ± 5.05 ng/mL, respectively). Serum iron binding capacity in these women was increased and reached 73.61 ± 5.68 $\mu\text{mol/L}$, and in healthy women, it was 53.0 ± 2.6 $\mu\text{mol/L}$.

According to the ESC results in women of reproductive age with IDA, this parameter was 1.2 times higher (19%) compared to the control group. The development of anemia is associated with significant changes in the osmotic stability of the erythrocytes, which results in an increase in the proportion of both unstable and high-resistance erythrocytes. This is reflected in changes in the pattern of the erythrocyte osmotic stability curve.

5. REFERENCES:

1. Akkaya, B., Kucukal, E., Little, J.A., Gurkan, U.A. *Biochimica et Biophysica Acta – Biomembranes*, **2019**, 1861(6), 1162-1171.
2. Alshalani, A., Acker, J.P. *Cryobiology*, **2017**, 76, 51-58.
3. Alshalani, A., Howell, A., Acker, J.P. *Cryobiology*, **2018**, 80, 30-37.
4. AlZahrani, K., Al-Sewaidan, H.A. *Indian Journal of Hematology and Blood Transfusion*, **2017**, 33(1), 109-115.
5. Burkibaiev, J.K., Abdrakhmanova, S.A., Savchuk, T.N., Zhiburt, E.B. *Klinicheskaya Laboratornaya Diagnostika*, **2017**, 3, 154-156.
6. Chowdhury, A., Waghmare, D., Dasgupta, R., Majumder, S.K. *Journal of Biophotonics*, **2018**, 11(8), e201700222.
7. Ciana, A., Achilli, C., Gaur, A., Minetti, G. *Cellular Physiology and Biochemistry*, **2017**, 42(3), 1127-1138.
8. Das, U., Saha, T., Das, S.K. *Indian Journal of Biochemistry and Biophysics*, **2018**, 55(5), 321-327.
9. Eapen, C.E., Ramakrishna, B., Balasubramanian, K.A. *Indian Journal of Medical Research*, **2018**, 147(April), 334-336.
10. Gallo, G., Bruno, R., Taranto, A., Martino, G. *Pharmacognosy Magazine*, **2017**, 13(51), S561-S566.
11. Gou, Z., Ruan, X., Huang, F., Fu, X. *Computer Methods in Biomechanics and Biomedical Engineering*, **2019**, 22(5), 465-474.
12. Grzeskowiak, L.E., Qassim, A., Grivell, R.M. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, **2018**, 58(6), E32-E33.
13. He, Y., Jia, S., Dewan, R.K., Liao, N. *Gene*, **2017**, 627, 556-562.
14. Kamyshnikov, V.S. Determination of permeability of erythrocyte membranes of OSE (osmotic resistance of erythrocytes), in *Handbook of Clinical and Biochemical Laboratory Diagnostics. Volume 2* (pp. 45-50), Minsk: Izdatel'skiy Dom Belyy Veter, **2003**.
15. Kanuri, G., Chichula, D., Sawhney, R., Kuriakose, K., De'Souza, S., Pais, F., Arumugam, K., Shet, A.S. *Haematologica*, **2018**, 103(12), 1991-1996.
16. Klichkhanov, N.K., Dzhaferova, A.M. *Biophysics (Russian Federation)*, **2018**, 63(4), 526-536.
17. Konovodova, Ye.N., Yakunina, N.A. *Iron Deficiency and Pregnancy*, **2010**. https://www.rmj.ru/articles/ginekologiya/Ghelezodeficitnye_sostoyaniya_i_beremennosty/, accessed May, 2019.
18. Kopytova, T.V. *Clinical Laboratory Diagnostics*, **2006**, 1, 18-19.
19. Kotelnikova, L.V. *The Metabolic Status of Erythrocytes and Its Correction with Stabilizers of Cell Membranes in Violation of Energy Homeostasis*, Kursk: Kursk State Medical University, **2000**.
20. Malkoch, A.V., Anastasevich, L.A., Filatova, N.N. *Reproductive Endocrinology*, **2013**, 5/13, 22-27.
21. Minetti, G., Achilli, C., Perotti, C., Ciana, A. *Frontiers in Physiology*, **2018**, 9(MAR), 286.
22. Nobre, C.S., Silva, J.A., Jácomo, R.H., Nery, L.F.A., Barra, G.B. *Cytometry Part B - Clinical Cytometry*, **2018**, 94(6), 910-917.
23. Prokopenko, L.G., Konoplya A.I., Laskova I.L. *Erythrocytes and Metabolic Immunomodulation*, Kursk: KSMU, **1995**.
24. Sawyer, A.A., Wise, L., Ghosh, S., Bhatia, J., Stansfield, B.K. *Transfusion*, **2018**, 58(5), 1327-1328.
25. Sutil-Vega, M., Rizzo, M., Martínez-Rubio, A. *Echocardiography*, **2019**, 36(3), 585-594.
26. Tikhomirov, A.L., Kocharyan, A.A. *Gynecology*, **2006**, 8(2), 44-47.
27. Tong, Z.-X., Chen, X., He, Y.-L., Liao, X.-B. *Physica A: Statistical Mechanics and its Applications*, **2018**, 509, 1183-1194.
28. Vazquez-Martin, A., Anatskaya, O.V., Giuliani, A., Erenpreisa, J., Huang, S., Salmina, K., Inashkina, I., Huna, A., Nikolsky, N.N., Vinogradov, A.E. *Oncotarget*, **2016**, 7(46), 75235-75260. DOI: 10.18632/oncotarget.12118.
29. Vinogradov, A.E., Shilina, M.A., Anatskaya, O.V., Alekseenko, L.L., Fridlyanskaya, I.I., Krasnenko, A., Kim, A., Korostin, D., Ilynsky, V., Elmuratov, A., Tsyganov, O., Grinchuk, T.M., Nikolsky, N.N. *Stem Cells International*, **2017**,

- 2362630, 1-14. DOI:
<https://doi.org/10.1155/2017/2362630>.
30. Vorobyeva, A.I. *Hematology Manual. Volume 2*, Moscow: Meditsina, **1985**.
31. WHO: The World Health Report. Reducing Risks, Promoting Healthy Life, **2002**.
<https://www.who.int/whr/2002/en/>,
 accessed May, 2019.

Table 1. Iron exchange parameters in the study groups

Parameters	Control group (n=20)	Patients with IDA (n=30)	p
HGB, g/L	132.1 ± 1.8	101.9±4.3	0.000
RBC, 10 ⁶ /mL	4.4 ± 0.08	4.1±0.1	0.269
MCV, fL	85.3 ± 0.9	73.2±2.1	0.004
MCH, pg	29.6 ± 0.2	22.8 ± 1.28	0.000
MCHC, pg	333.96 ± 7.76	315 ± 13.19	0.385
SI, µmol/L	15.37 ± 1.1	10.1 ± 2.0	0.019
TSIBC, µm/L	53.0 ± 2.6	73.61 ± 5.68	0.027
Ferritin, ng/mL	86.81 ± 5.05	31.21 ± 5;3	0.000

Table 2. Erythrocyte sorption capacity in women with IDA

Parameter, unit	Healthy women (n=20)	Women with IDA (n=30)
ESC, %	23.6 ±1.3	38.2±1.2*

Note: * significance of differences compared to the controls, p<0.01

Table 3. Osmotic stability of the erythrocytes in healthy women and women with anemia, % (M±m)

Group	Urea solution concentration						
	33%	38%	44%	50%	55%	61%	100%
Control (n =20)	0.11 ±0.05	0.135 ±0.038	0.33 ±0.06	1.04 ±0.99	1.50 ±0.05	1.63 ±0.045	1.66 ±0.07
I stage IDA (n = 22)	0.77 ±0.14*	0.58 ±0.13*	0.31 ±0.085	0.41 ±0.10	0.695 ±0.14	0.72 ±0.15*	1.69 ±0.02
II stage IDA (n = 8)	0.65 ±0.52	0.44 ±0.22	0.28 ±0.1	0.32 ±0.057	0.47 ±0.07*	0.58 ±0.25*	1.48 ±0.09

Note: * significance of differences compared to the controls, p<0.01

Figure 1. Osmotic stability curve