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EVALUATION OF THE ANTI-ADIPOSE AND ANTIOXIDANT EFFECTS OF LACTOSERUM ZONAR IN AN EXPERIMENTAL MODEL OF OBESITY-INDUCED IN SPRAGUE-DAWLEY RATS

EVALUAREA EFECTELOR ANTIADIPOASE ȘI ANTIOXIDANTE ALE LACTOSERUMULUI ZONAR ÎNTR-UN MODEL EXPERIMENTAL DE OBEZITATE INDUSĂ LA ȘOBOLANII SPRAGUE-DAWLEY

Amalia Marina NEAGU[1)], Sandra ANDREI[1)], H. RAFA[1)], Alexandra Iulia DREANCĂ[1),*)], Raluca MARICA[1)], Alina-Elena PÂRVU[2)], Mărioara MOLDOVAN[3)], I. MARCUS[1),]

ABSTRACT | REZUMAT

The study aimed to evaluate the anti-adipose and antioxidant effects of the lactoserum Zonar in an experimental model of obesity in Sprague-Dawley rats. The study was performed on 30 healthy adult rats of the Sprague-Dawley breed, males aged 3 months, weighing on average 450 ± 35 g. The groups of animals used in the experiment were structured as follows: healthy control group-MS (n = 5); obese control group -MO (n = 5); standard food and lactoserum Zonar group-HSZN (n = 5); hyperlipidic food and lactoserum Zonar group-HHZN (n = 5); Obesity 1 group -OB 1 (n = 5) and obesity 2 group -OB 2 (n = 5). Lactoserum Zonar had the ability to prevent the onset of insulin resistance. In addition, it had antioxidant action, demonstrated by the low level of oxidative stress markers in the experimental groups supplemented with it. It was also observed that it had a protective effect on hepatocytes, preventing the accumulation of triglycerides in the cytoplasm. Considering the results obtained by oxidative parameters, it can be stated that lactoserum Zonar is neither hepatotoxic nor nephrotoxic, having even a slight antioxidant action on the kidneys. Also, lactoserum Zonar prevents the accumulation of triglycerides in adipocytes, an effect observed in all supplemented groups both as a preventive and palliative form.

Keywords: lactoserum Zonar, obesity, rats, HOMA-IR, oxidative stress

Scopul studiului a fost de a evalua efectele antiadipoase și antioxidante ale lactoserului Zonar într-un model experimental de obezitate la șobolani Sprague-Dawley. Studiul a fost efectuat pe un număr de 30 de șobolani, adulți, sănătoși din rasa Sprague-Dawley, masculi, în vârstă de 3 luni, cu o greutate medie de 450 ± 35 g. Grupele de animale utilizate în experiment au fost structurate după cum urmează: grup control MS (n=5); grup control obez-MO (n = 5); hrana standard si lactoser Zonar-HSZN (n=5); hrana hiperlipidică și lactoser Zonar-HHZN (n = 5); Obezitate 1 grup -OB 1 (n=5) și obezitate 2 grup -OB 2 (n=5). Lactoserul Zonar reușește să prevină apariția sindromului de rezistență la insulină. A avut, de asemenea, acțiune antioxidantă, demonstrată printr-o diminuare considerabilă a markerilor de stres oxidativ în grupurile experimentale care au fost suplimentate cu Zonar. S-a observat că acesta a avut un efect protector asupra hepatocitelor, prevenind acumularea trigliceridelor în citoplasmă. Lactoserul Zonar nu prezintă hepatoxicitate sau nefrotoxicitate, având acțiune antioxidantă sistemică. De asemenea, loturile suplimentate atât ca formă preventivă, cât și paliativă cu lactoser, nu prezintă procese distrofice asociate cu acumularea trigliceridelor în adipocite, dovedindu-și astfel capacitățile preventive în obezitate și complicațiile acesteia.

Cuvinte cheie: lactoser Zonar, obezitate, șobolani, HOMA-IR, stres oxidativ

The adipose tissue stores energy in the form of lipids and controls their mobilisation and distribution in the body, being directly involved in metabolic regulation. The storage of triglycerides (TG) in adipocytes over a long period leads to an increase in size (4). Triglycerides that are stored in adipocytes are broken down into glycerol and lipolytic fatty acids, a phenomenon that occurs following caloric restriction. Studies have shown that excess adipose tissue (excess fat) can cause metabolic abnormalities such as dyslipidemia and insulin resistance. It can also increase the risk of cardiovascular complications, such as coronary heart disease and high blood pressure (20;6). Normally, adipose tissue represents between 10 and 25% of body weight, and when this body mass index (BMI) exceeds 30 kg /m^2 it represents a major health risk (13). Milk provides a wide range of biologically active components, such as bioactive proteins and peptides, oligosaccharides, immunoglobulins, and fats / lipids that can protect against pathogens if consumed regularly. The biological properties of whey proteins are widely recognized and have been increasingly explored by various industries in scientific research studies and food applications.

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
2) University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, Romania
3) Institute of Chemistry "Raluca Ripan", Cluj-Napoca, Romania
*) *Corresponding author: alexandra.dreanca@usamvcluj.ro*

After ingestion, whey is hydrolysed into bioactive peptides and amino acids detected by the nervous system at various levels of the gastrointestinal tract and central nervous system. These detection systems acts through satiety signals by reducing the amount of food consumed (11). Some models of the induction of obesity in rats are found in the international literature. The main experimental models of inducing obesity are: by lesion of the ventromedial hypothalamic nucleus (VMH), which can be achieved mainly in two ways (administration of monosodium glutamate or direct electrical injury); ovariectomy; administration of hypercaloric diets; and genetic manipulation for obesity (26). The research aimed to evaluate the bioactive nutraceutical effects of Zonar lactoserum used as a food supplement on obesity induced by a hyperlipidic diet in Sprague-Dawley rats.

MATERIALS AND METHODS

The study was performed on 30 healthy adult rats of the Sprague-Dawley breed, males aged 3 months, weighing on average 450 ± 35 g. The animals were raised in the Biobase of the Faculty of Veterinary Medicine, USAMV Cluj-Napoca, in compliance with the standard conditions: temperature 22-23ºC, humidity 55% and a photoperiod of 12h light / 12h dark. The animals were fed standard granulated feed for rodents (provided by the Cantacuzino Institute, Bucharest, Romania) and water ad libitum. The experimental protocol was approved by the Research Ethics Commission of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and was authorised by DSVSA Cluj (Decision no. 48 / 29.03.2017). The groups of animals used in the experiment were structured as follows: healthy control group-MS (n = 5); obese control group-MO (n = 5); standard food and lactoserum Zonar group-HSZN (n = 5); hyperlipidic food and lactoserum Zonar group-HHZN (n = 5); Obesity 1 group - OB 1 (n = 5) and obesity 2 group - OB 2 (n = 5). In the case of the OB 1 and OB 2 groups, the experimental protocol was divided into two stages. Respectively, in stage 1, obesity was induced in both groups for 7 weeks, and in stage 2, group OB 1 switched to a standard diet and lactoserum Zonar, and OB 2 group still remained on hyperlipidic diet and just lactoserum Zonar was introduced. Neagu et al. (2020) first published the experimental protocol. The animals received two types of combined granular food, provided by the Cantacuzino Institute, Bucharest; the structure of the standard food ration (combined food) contained of: 18% protein, 1.5% fat, 5% fibre, and the hyperlipidic food composition is 21 % protein, 16% fat, and 3.5% fibre. During the experiment, a commercial formula of sweet lactoserum was administered to animals, produced and marketed under the name of lactoserum Zonar by SC EmbrionSRL, Satu Mare, Romania (Table 1). Lactoserum Zonar was characterised in terms of physico-chemical composition.

The haematology and biochemistry methods

The blood count was performed using the automatic analyser Abacus Junior Vet 5 Diff. Leukocyte parameters (total number of leukocytes, lymphocytes, average cells, granulocytes), erythrocyte parameters (total number of red blood cells, haematocrit, and haemoglobin), and platelet parameters (total number of platelets) were monitored. The biochemical Insulin (Ultrasensitive Mercody Rat Insulin ELISA, 10-1252-01 / lot 27126) was dosed by ELISA method with the Microplate Photometer device, type MPP-96.

Evaluation of the homeostatic model of insulin resistance (HOMA2-IR, HOMA-S%, HOMA-β%)

The Homeostatic model assessment is a method used to quantify insulin resistance and beta cell function. It was first described as HOMA by Matthews et al. in 1985. The HOMA2-IR index was obtained by the programme HOMA Calculator v2.2.3 downloaded from the web page at https://www.dtu.ox.ac.uk/homacalculator/download.php

Determination of tissue oxidative stress parameters

The Protein extracts from liver and kidney were obtained using potassium phosphate buffer (pH=7; 35). TAC activities, lipid peroxidation, and protein oxidation were estimated in this protein extract using photometric methods. Antioxidant enzyme activity was determined in the liver and kidney tissues of all experimental groups using commercial kits (Randox). The degree of lipid oxidation was determined by measuring the loss of unsaturated fatty acids; the amounts of primary peroxidation products. One of the most frequently applied tests is the thiobarbituric acid (TBA) test. The method is based on the reaction of TBA with malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation (21).

Determination of organ-somatic index (weight of organs and relative weight of organs)

The liver and kidneys were carefully dissected and weighed at the end of the experiment. These organs were then stored in 10% formaldehyde. Organ somatic index (relative organ weight) was calculated using the formulas

Table 1

Physico-chemical composition of Lactoserum Zonar
(Zonar lactoserum order note no. 4 / 01.05.2017)

Physico-chemical characteristics						
Indices	pH	Acidity (°T)	Acidity index (mg/g)	Dry substances (%)	Water (%)	Sodium chloride (%)
	6.2	10	0.38	6.2	93.8	0.2

MINERAL COMPOSITION (mg/kg)									
Ca	K	Mg	Na	Cu	Fe	Pb	Zn	P	Ca/P
228.9	639.4	56.9	53.5	0.2	1.34	0.01	0.5	363.1	0.63

HPLC-UV analysis of proteins (mg/g)						
Total protein	BSA	α Lactalbumin	β Lact-globulin B	β Lact-globulin A	Carbohydrates (mg/100g)	Lactose
2.42	0.111	1.108	0.77	0.431		48

described by Vani et al.2000.

$$\text{Relative organ weight (\%)} = \frac{\text{current organ weight (g)} \times 100}{\text{weight of the animal on the day of euthanasia (g)}}$$

Histopathologic measurements

For histopathologic determination, abdominal adipose tissue, liver, kidney, and pancreas were used. The collected samples were immersed immediately after collection in a 10% formaldehyde solution at laboratory temperature for fixation, and then they were processed for inclusion in paraffin. The tissues thus prepared were sectioned with the help of the Leica microtome at a thickness of 5 µm, and stained using the standard Haematoxylin-Eosin staining. The histopathological examination was carried out using the Olympus BX51 microscope, connected to the Olympus DP-25 digital camera, with which the histopathological images were taken. The morphometric measurements were performed using the "Cell B soft" programme.

Statistical processing of the results

Data collection and processing were done using Microsoft Office 2010 programmes, and graphics processing was done in SPSS version 21. All data are reported as mean ± standard deviation. Differences between experimental and control conditions were analysed using the T-test. Statistical significance was set at $p < 0.05$ (95% confidence interval). The evaluation of the differences in the values with normal distribution was carried out by the one-way ANOVA multivariate analysis test ($F (5.24) = 7.697$, $p = 0.000$), followed by a Post Hoc Tukey test, which demonstrated that the individuals in the MO group (control positive) show statistically significantly higher values than those in the MS group ($p = 0.005$).

RESULTS AND DISCUSSIONS

Determination of haematological and biochemical profiles and evaluation of the HOMA-IR index in rats

The haematological results showed no statistically significant difference in rats fed a hyperlipidic diet compared to rats fed a standard diet. Shawky (2015) obtained similar results to those obtained in our study, which argued that there is insufficient data available regarding the effect of a hyperlipidic diet on haematological parameters. Instead, Kilany et al. (2020) obtained neutrophilia and lymphopenia in the groups fed a hypercaloric diet. Neutrophilia in obese rats is thought to be due to the fact that obesity can induce the production of glucocorticoids and IL-6, which play a role in bone marrow granulopoiesis, and lymphocytopenia in obese rats is a common finding during the systemic inflammatory response due to the reduction of T cells in the blood peripheral, spleen, and thymus. The difference between the results obtained in our study and the study by Kilany et al. (2020) may be due to the experimental period, which was 20 weeks compared to the 11 weeks in our study, and the food administered to the rats in that study additionally contained 5.14% sucrose. All the experimental groups in our study (HSZN, HHZN, OB1, and OB2) that were supplemented with Lactoserum Zonar, both in preventive and palliative forms, did not show statistical changes in haematological parameters compared to the MO and MS groups (Tables 2 and 3). The level of leukocytes and lymphocytes in this study was slightly decreased in the HSZN group, where the lower value of leukocytes may be due to the effect of AA found in lactoserum, such as lysine, which, by exceeding the volume required by the body, can affect intracellular depletion of arginine and thus cause a decrease in the number of leukocytes. Also, food restriction before blood sampling can influence the lower plasma concentration level of arginine with its overexpression in the small intestine and the stress hormone cortisol (2).

From a medical point of view, an increase in the num-

Table 2

Leukogram values at the end of the experiment (n = 5)

Groups	**WBC** (10^9/l)	**LYM** (10^9/l)	**MON** (10^9/l)	**NEU** (10^9/l)
MS (n = 5)	7.81 ± 3.47	5.8 ±2.32	0.53 ± 0.39	1.47 ±0.80
MO (n = 5)	7.43 ± 2.32	5.90 ± 1.71	0.29 ± 0.39	1.23 ± 0.62
HSZN (n = 5)	4.01±2.43	3.08±1.36	0.25±0.38	0.66±0.84
HHZN (n = 5)	7.52 ± 4.85	6.29 ± 4.13	0.17 ± 0.14	1.05 ± 0.67
OB1 (n = 5)	7.20±2.50	5.97±2.19	0.16±0.11	1.06±0.36
OB2 (n = 5)	8.02±2.92	6.82±2.58	0.18±0.12	1.01±0.41

Table 3

Erythrogram and thrombogram values at the end of the experiment (n = 5)

Groups	**RBC** (10^{12}/l)	**HGB** (g/l)	**HCT** (%	**PLT** (10^9/l)
MS (n = 5)	7.25 ±0.80	14.66 ± 1.95	36.53 ± 3.59	630 ± 346.17
MO (n = 5)	7.53 ±0.54	14.18 ± 1.19	37.87 ± 2.38	485.4 ± 291.73
HSZN (n = 5)	6.11 ± 1.20	14.5 ± 2.62	35.07 ± 6.77	274.6.8 ± 262.18
HHZN (n = 5)	6.91±1.29	14.12±2.19	37.21±6.11	478.4±313.79
OB1 (n = 5)	7.66±0.79	13.82±1.27	39.73±3.90	348.6±230.9
OB2 (n = 5)	7.65±0.41	14.46±0.23	40.05±0.89	671.4±52.02

ber of leukocytes normally indicates an infection or inflammation. In this study, there was no increase in the number of leukocytes, so we can say that there was no inflammation or infection throughout the entire experiment. The effects of lactoserum proteins benefit animal health in the blood system. Several previous studies have successfully demonstrated the impact of different forms of whey protein (isolate or whey protein) on improving the function of the blood system and reducing the risk of hypertension and metabolic syndrome (2). It can be suggested that the administration of lactoserum Zonar in rats does not produce haematological changes regardless of the type of diet administered, with all values falling within the normal limits provided by the device for the species, sex and age of the animals. Our results are similar to the experiment performed by Andoyo et al., 2021.

Determination of the Homeostatic Model Assessment (HOMA-IR)

It is a validated method to measure insulin resistance using (fasting) glucose and insulin parameters. The original HOMA1-IR model was first published by Matthews et al. (1985) and has been widely used, especially in clinical and epidemiological studies (8). Recently, the model has been updated with some physiological adjustments to a computer version (HOMA2-IR) and thus provides a more accurate index. The stand-alone version of the HOMA2 calculator additionally uses the model to estimate beta-cell function (HOMA_%B) and insulin sensitivity (HOMA_%S). Specific insulin or C-peptide (fasting) values can be used (https://www.dtu.ox.ac.uk/homacalculator/download.php).

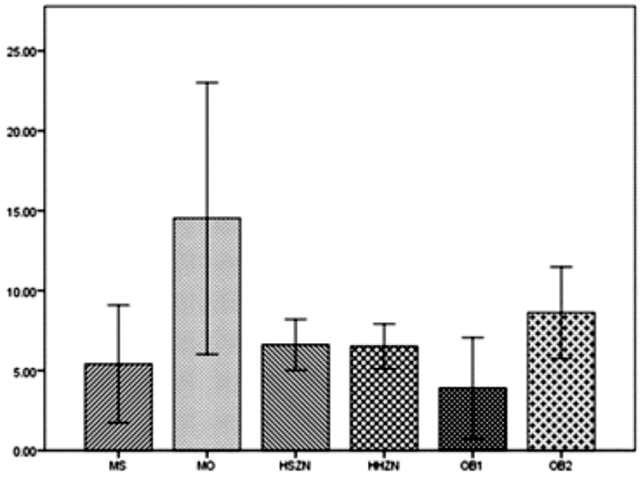

Fig. 1. Serum Insulin values in the experimental groups (mean ± SD). (n = 5; one-way ANOVA, Tukey post hoc test). *p <0.05; ** p <0.01 against the MO group

We have a statistically significant increase in the level of insulin in the MO group (14.51±6.84 µU/ml) compared to the MS (5.41±2.96 µU/ml; <0.01), HSZN (6.61±1.28 µU/ml; <0.05), HHZN (6.51±1.12 µU/ml; <0.05), and OB1 groups (4.60±1.56 µU/ml; <0.01). On the other hand, the insulin level in the OB2 group (8.61±2.30 µU/ml) did not show any statistically relevant change. However, an increase in the insulin level is still observable compared to the other groups in the study (Fig. 1).

The glucose values used in calculating the HOMA indexes were published in a previous article by Neagu et al. (2020). Insulin resistance is expressed as HOMA-IR indices; the higher the value, the greater the insulin resistance. Reference values for the index (www.theblood code.com).

Table 4

Calculation of HOMA-IR, S, and β Index based on fasting insulin and glucose (mean ± SD)
(n = 5; one-way ANOVA, Tukey post hoc test)

Parameters groups	HOMA-IR	HOMA-S%	HOMA-β%
MS (n = 5)	0.95±0.45***	126.86±60.45**#	31.69±21.57
MO (n = 5)	2.88±1.21	39.96±16.61	32.58±14.93
HSZN (n = 5)	1.14±0.24**	90.08±16.46	39.88±19.09
HHZN (n = 5)	1.13±0.20**	90.56±16.76	34.40±4.73
OB1 (n = 5)	0.85±0.30***	130.36±48.25**#	20.42±6.67
OB2 (n = 5)	1.73±0.48*	61.84±19.11	22.46±6.25

HOMA2-IR is presented as follows: <1 insulin sensitivity, which is optimal; >1.9 early insulin resistance; >2.9 insulin resistance (8). The following results were obtained: the MO group had the highest HOMA-IR index (2.88±1.21) compared to the MS group (0.95±0.45; <0.001), so we can say that the animals from the MO group developed insulin resistance (Table 4). Similar results were also obtained by Rojas et al. (2018), where, like in our study, the HOMA-IR index was increased in groups that received hyperlipidic food. The results obtained in the groups supplemented with lactoserum Zonar, HSZN (1.14±0.24; <0.01), HHZN (1.13±0.20; <0.01), OB1 (0.85±0.30; <0.001), and OB2 (1.73± 0.48; <0.05) groups were with an optimal index level compared to the MO group (Table 4). Insulin sensitivity is expressed as HOMA%-S; the higher the value, the greater the subject's insulin sensitivity (1). The HOMA-S% index is calculated to predict the success rate of the therapy used. The determination is used, in diabetic subjects (type 2), to see if the therapy is working or not (10).

The MO group (39.96±16.61%) had the lowest insulin sensitivity index compared to the MS group (126.86± 60.45%; <0.01) and the OB1 group (130.36±48.25%; <0.01). Also, the OB2 group (61.84±19.11%) had a statistically lower index compared to the MS group (126.86±60.45%; <0.05) and OB1 group (130.36 ±48.25%; <0.05). Thus, in the case of our study, the groups that received Lactoserum Zonar had higher percentage values than the MO group, with the OB1 group having the highest value, suggesting that switching from a hyperlipidic diet to a standard diet and Lactoserum Zonar had the greatest impact on insulin sensitivity.

Table 5

Determination of oxidative stress markers and antioxidant capacity in LIVER (mean ± SD).
(n = 5; one-way ANOVA, test post hoc Tukey)

Group/ tissue Liver	Protein (mg/ml)	TAC (nmol/mg)	MDA (nmol/mg)
MS (n = 5)	4.20±0.83	0.14±0.02	7.00±0.68
MO (n = 5)	5.96±2.34	0.27±0.39	7.39±2.66
HSZN(n = 5)	5.20±0.83	0.24±0.02	6.67±0.15
HHZN(n = 5)	4.19±1.52	0.07±0.04	10.36±5.09
OB1 (n = 5)	5.28±2.08	2.30±2.25	6.16±1.61

Table 6

Determination of oxidative stress markers and antioxidant capacity in KIDNEY (mean ± SD). (n = 5; one-way ANOVA, test post hoc Tukey)

Group/ tissue Kidney	Protein (mg/ml)	TAC (nmol/mg)	MDA (nmol/mg)
MS (n = 5)	3.29±0.21	1.15±0.06	9.43±0.57
MO (n = 5)	5.88±0.13	0.98±0.09	9.81±0.80
HSZN (n = 5)	3.62±0.75	1.25±0.07*	9.47±0.60
HHZN (n = 5)	4.26±1.13	1.30±0.19*	10.57±2.76
OB1 (n = 5)	4.52±0.03	0.58±0.40	9.08±0.05
OB2 (n = 5)	4.78±0.94	1.35±0.14*	8.84±1.30

The secretory capacity of beta cells is expressed as HOMA-β%; the higher the value, the more insulin secretory capacity beta cells have to cope with blood glucose levels (1). As for the HOMA-β% index, it showed no changes from a statistical point of view in any of the experimental groups (Table 4). Our study agrees with the results of the HOMA-IR index and with the results found in the literature that excess fat plays a significant role in developing insulin resistance in humans and rodents (19). However, animals fed a hyperlipidic diet never developed diabetes, which is consistent with previous findings in the Sprague-Dawley rats, and it is attributed to the compensatory increases in both pancreatic β-cell mass and glucose-stimulated insulin secretion (19). Similar to our study, Pal et al. (2010) demonstrated that lactose supplementation reduced insulin levels and increased insulin sensitivity (HOMA index). In our study, we obtained the best results in the OB1 group, where the caloric restriction imposed by changing the food from a hyperlipidic one to a standard food and supplementing with lactoserum Zonar had the best results by reducing insulin levels and increasing sensitivity compared to the MO group.

Table 7

Effects of Lactoserum Zonar on weights and relative weights (organo-somatic index) of rat organs after the experimental period (mean ± SD); (n= 5; one-way ANOVA, test post hoc Tukey, ** p <0.01 against MO group)

Groups	Liver (g)	Liver (%)	Kidney (g)	Kidney (%)
MS (n = 5)	17.13±1.48	2.84±0.24	3.36±0.64	0.57±0.12
MO (n = 5)	17.32±2.09	2.59±0.28	3.32±0.45	0.49±0.06
HSZN (n = 5)	13.58±1.38**	2.52±0.22	3.05±0.36	0.56±0.04
HHZN(n = 5)	17.26±1.63	2.86±0.39	3.55±0.42	0.59±0.10
OB1 (n = 5)	14.54±2.39	2.61±0.59	3.29±0.23	0.59±0.10
OB2 (n = 5)	16.20±1.05	2.89±0.40	3.35±0.41	0.60±0.12

Determination of the antioxidant effects of lactoserum Zonar by dosing the oxidative stress markers in tissue organs. Oxidative stress is defined as a dynamic imbalance between the amounts of reactive oxygen species (ROS) generated in the body and the level of antioxidants (AO) that can protect the body against harmful effects (9). The tissue samples examined in our study were kidney and liver, where, after the initial determination of protein loads, the following markers of oxidative stress were determined: malondialdehyde (MDA), which shows the degree of lipid peroxidation, and the total tissue antioxidant capacity (TAC). The obtained results of our study showed a statistical antioxidant increase in renal TAC in the HSZN group (1.25±0.07 nmol/mg), the HHZN group (1.30±0.19 nmol/mg), and the OB2 group (1.35±0.14 nmol/mg) compared to the MO group (0.58±0.40 nmol/mg) ($p<0.05$), due to the consumption of lactoserum Zonar (Table 6). The antioxidant effects of Lactoserum Zonar may be due to various natural antioxidant compounds such as beta-lactaglobulin, alpha-lactalbumin, immunoglobulins, bovine serum albumin, lactaferrin, and/or lactoperoxidase, etc. The TAC values determined from the liver did not show any statistical change. No statistical difference in the MDA marker was observed in any experimental group in both the liver and kidney (Tables 5 and 6). Obesity is characterised not only by an increase in body weight but also by changes in body composition, especially an increase in body fat (3). In the values obtained following organ weighing, only the HSZN group had a statistically significantly lower liver weight (13.58±1.38 g; <0.01) compared to the MO group (17.13±1.48 g) and without any change in the organo-somatic index. Hong et al. (2015) had similar results to our study: they statistically significantly reduced the liver weight in the group that received fermented whey. Even in the case of kidney weighing (weight and organo-somatic index), there were no significant statistical changes in any of the experimental groups. Considering the results obtained in our study, it can be stated that Lactoserum Zonar is neither hepatotoxic nor nephrotoxic, having even a slight antioxidant action at the renal level.

Histomorphometric (quantitative) analysis of adipose tissue

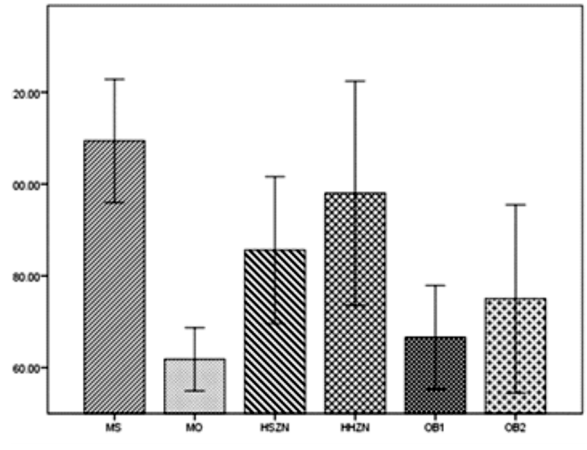

Fig. 2. Number of adipocytes in experimental groups (mean ± SD)

In the development of obesity, the expansion of adipose tissue is frequently based on adipocyte hypertrophy (an increase in adipocyte size), which is a known cellular stress factor for adipocytes, especially for the rough endoplasmic reticulum (25). Through the histomorphometric analysis of the adipocytes collected from the abdominal region of the animals in our study, it was observed that the MO group (61.8±5.54 adipocytes/field) had a statistically significantly lower number of adipocytes compared to the MS group (109.4±10.80 adipocytes/field; <0.001), as can be seen in Fig. 2. Regarding the diameter of the adipocytes, the following statistical changes were observed: the MO group (74.77±0.95 μm) presented the largest adipocyte sizes compared to the MS group (60.59±2.43 μm; <0.01). These results are due to an in-

crease in the volume of adipocytes following accumulation with triglycerides in the obese control group. The experimental groups that received preventive lactoserum Zonar had a higher number and a smaller diameter of adipocytes, HSZN (85.6±12.89 adipocytes/field; <0.05; (56.80±6.70 μm; <0.001), HHZN (98±19.65 adipocytes / field; <0.01; 57.36±3.68 μm; <0.001) compared to the MO group (Figs 2 and 3). Regarding the obese groups, group OB1 (66.6±9.09 adipocytes/field; <0.001) and OB2 (75±16.50 adipocytes/field; <0.01) who received lactoserum Zonar in palliative form had a statistically significant lower number of adipocytes than the MS group and without any statistical change compared to the MO group (Fig. 2). Adipocyte diameter in the obese groups that were supplemented with lactoserum Zonar, the OB1 group (63.74±7.34 μm; <0.05) had a statistically significant smaller diameter compared to the MO group (Fig. 3). The OB2 group (67.94±3.66 μm) had statistically significantly higher adipocyte diameter compared to the HSZN and HHZN groups (<0.05) (Fig. 3). Using the SPSS programme, we performed the Pearson correlation to determine the relationship between the number of cells/ microscopic field and their diameter (μm). Thus, we obtained a negative correlation between the number of cells /field and their diameter (μm), which was statistically significant (r = -.667, n = 30, p = .000), meaning that if the size of the cells in the field increases, their number decreases. Rojas et al. (2018) obtained results similar to ours regarding the group of rats fed a hypercaloric diet.

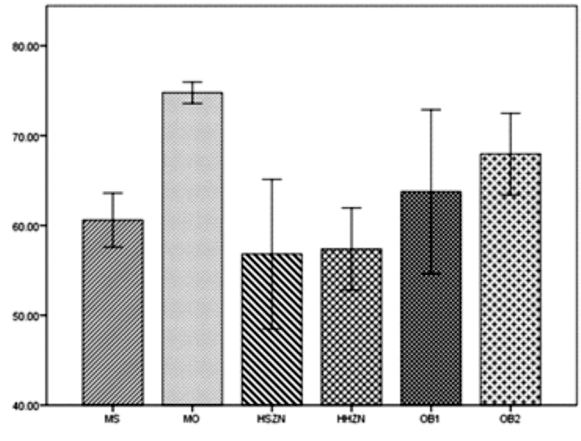

Fig. 3. Mean adipocyte area (μm) of the experimental groups (mean ± SD)

Our results are consistent with the results obtained by Liisberg et al. in 2016, where the samples collected from mice fed with a hyperprotein diet obtained from casein had a smaller adipocyte diameter in all experimental groups compared to the control group. This fact is also highlighted in other individuals who received hyperprotein diets, but the protein was obtained from other sources. It can be stated that lactoserum Zonar had anti-fat effects due to the reduced size and increased number of adipocytes in the experimental groups that received lactoserum compared to the MO group.

Qualitative histopathological analysis of adipose tissue

Regarding the histopathological appearance of the adipose tissue, differences were observed, especially between the MO group, where the animals received only hyperlipidic food without any other addition, and the groups in which the Zonar was also administered in addition to the hyperlipidic food or the standard food. The observable pathological change was represented by adipocyte hypertrophy. We can observe that in the samples obtained from individuals from the MO group, the adipocytes show an increase in volume due to the accumulation of triglycerides in their cytoplasm (Fig. 4). A slight hypertrophy of adipocytes can also be identified in the case of groups OB1 and OB2, their volume being increased compared to normal, but visibly lower than in individuals from the obese control group (Fig. 6). This result is due to the effect of the Zonar administered as a food supplement in both groups. Liisberg et al. (2016) obtained similar results to our study in histopathological appearance in mice with a hypercaloric diet compared to groups that were supplemented with casein. Rojas et al. (2018) had similar results to our study regarding the group that was subjected to hypercaloric diets, the histopathological aspects being adipocyte hypertrophy. The increase in volume of adipocytes occurs in response to excessive caloric intake and weight gain that is induced by long-term administration of hypercaloric food, where fat deposits lead to an increase in volume of adipocytes. As can be seen in Figures 5 and 6, the histopathological appearance of the adipocytes did not undergo significant changes in any of the samples collected from the adipose tissue; no inflammatory or cell necrosis aspects were observed; the only identifiable changes remained those of cell morphology.

According to Ellulu et al. (2017), inflammation was frequently associated with obesity. This is associated with hypertrophy, hyperplasia, cellular hypoxia, and adipocyte necrosis. Although it is well documented that the administration of hypercaloric food in rats induces inflammation in white fat deposits, in our study, the presence of inflammatory cell infiltrate in adipose tissues was not highlighted in any of the experimental groups. These results are consistent with the results obtained by Rojas et al. in 2018. Histopathological analysis of samples collected from the liver, pancreas, and kidneys. Microscopic examination of the liver revealed, in the MO group, which received only hyperlipidic food, as the predominant lesion, moderate microvacuolar hepatic steatosis, especially with Centrolobular localization (Fig. 7). It is possible to identify the presence of multiple lipid vacuoles (triglycerides) of variable sizes in the hepatocyte cytoplasm, with the nucleus moving to the periphery and in different stages of necrosis. Although hepatic steatosis was not evaluated quantitatively in the groups that received lactoserum Zonar in addition to food, this lesion is absent. The exception is the OB1 group, where early hepatic steatosis was identified (Fig. 9). Rojas et al. (2018) obtained results similar to those obtained in our study on microscopic examination of the liver, identifying minimal to moderate hepatocellular vacuolization, which was present in both sexes in the hypercaloric-fed groups. Likewise, there was no damage to the epithelium of the bile ducts or the presence of an inflammatory infiltrate in the experimental groups. From this, it follows that the administration of lactoserum Zonar has a protective effect on hepatocytes, preventing the accumulation of triglycerides in their cytoplasm and, therefore, preventing the appearance of pathological lesions at the level of the liver (Fig. 8).

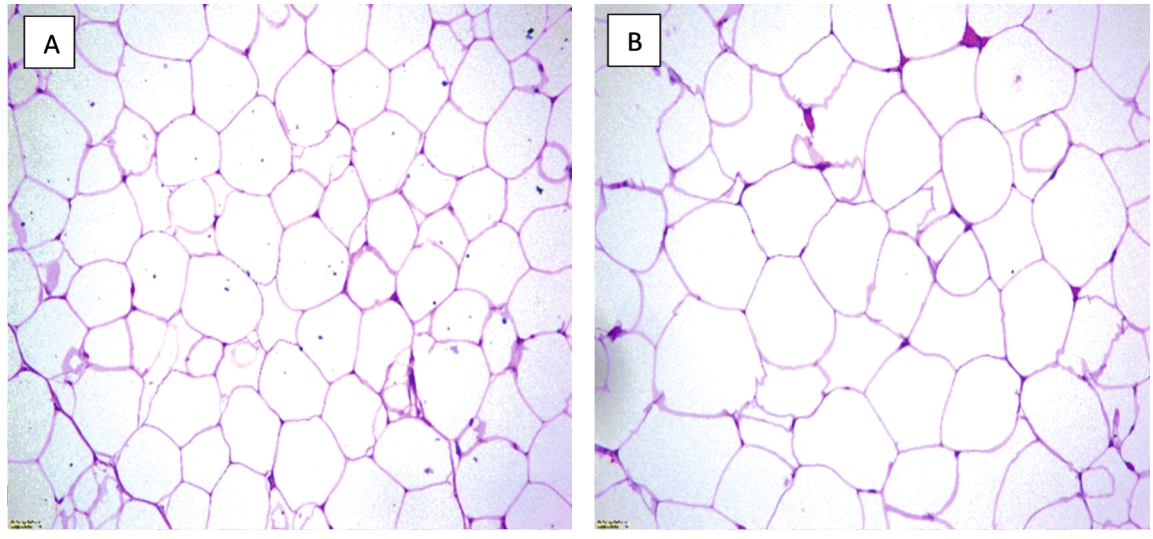

Fig. 4. Histological aspect of adipose tissue: A - MS; B – MO, (HE, obx20 µm)

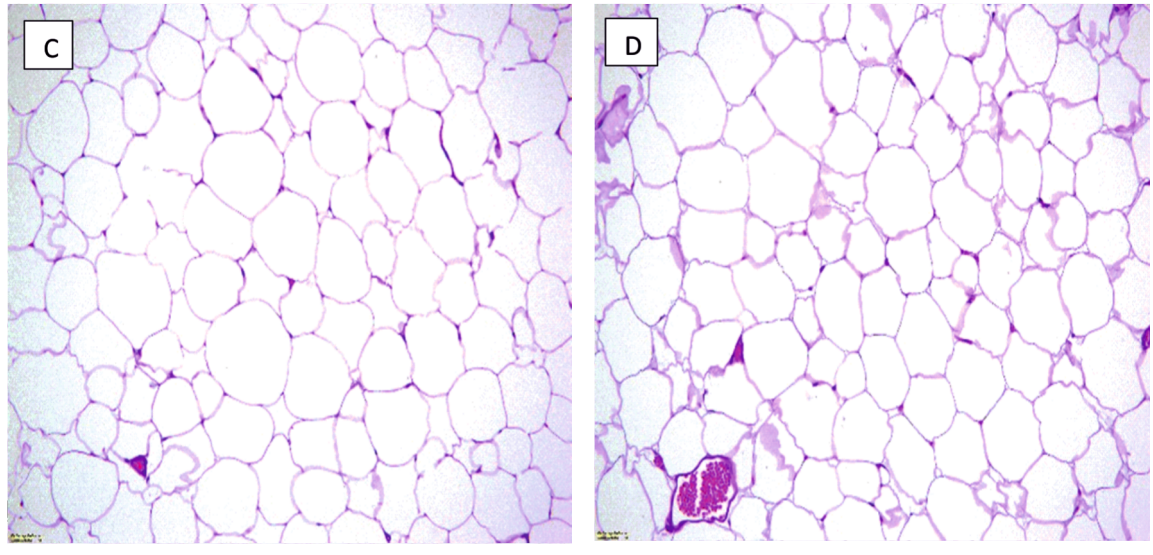

Fig. 5. Histological aspect of adipose tissue: C – HSZN; D – HHZN (HE, obx20 µm)

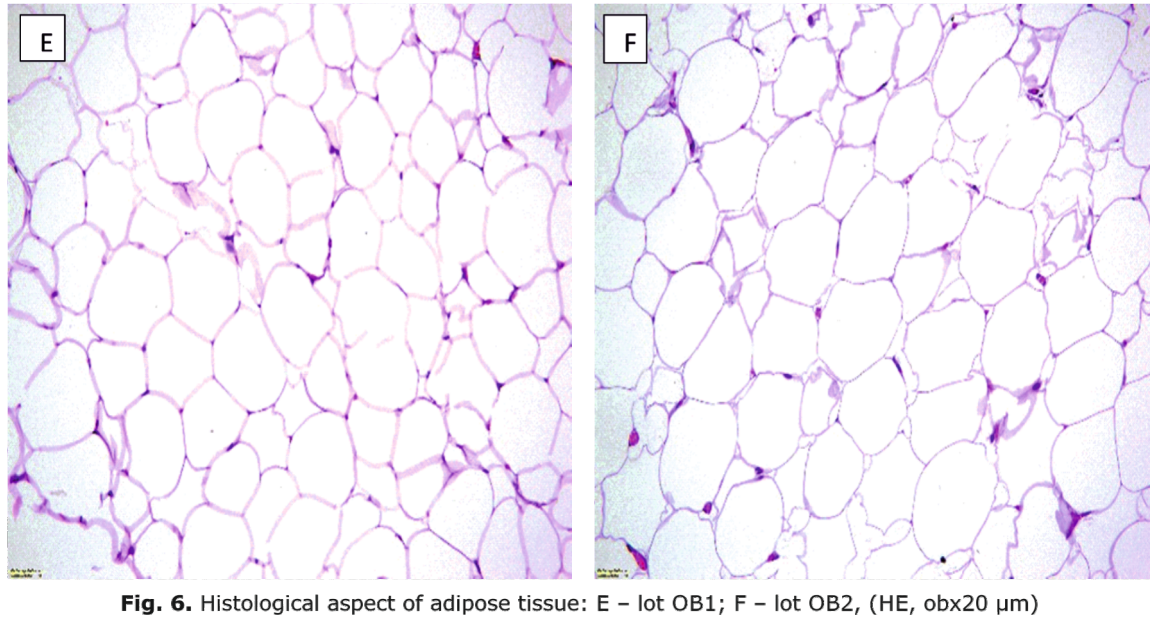

Fig. 6. Histological aspect of adipose tissue: E – lot OB1; F – lot OB2, (HE, obx20 µm)

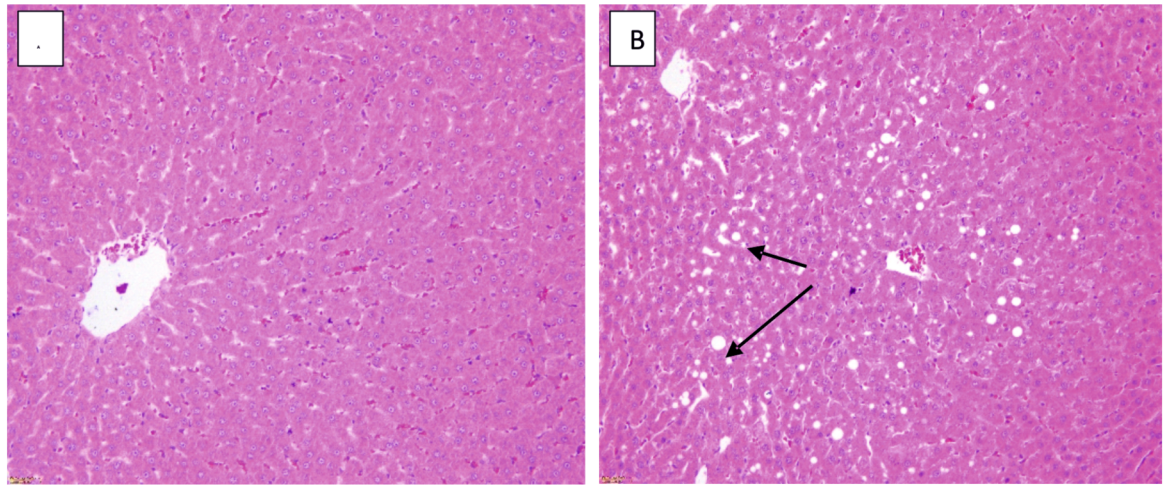

Fig. 7. Histological aspect of the liver: microvacuolar type (HE, ob x 20 µm)
A –MS; B – MO, moderate hepatic steatosis,

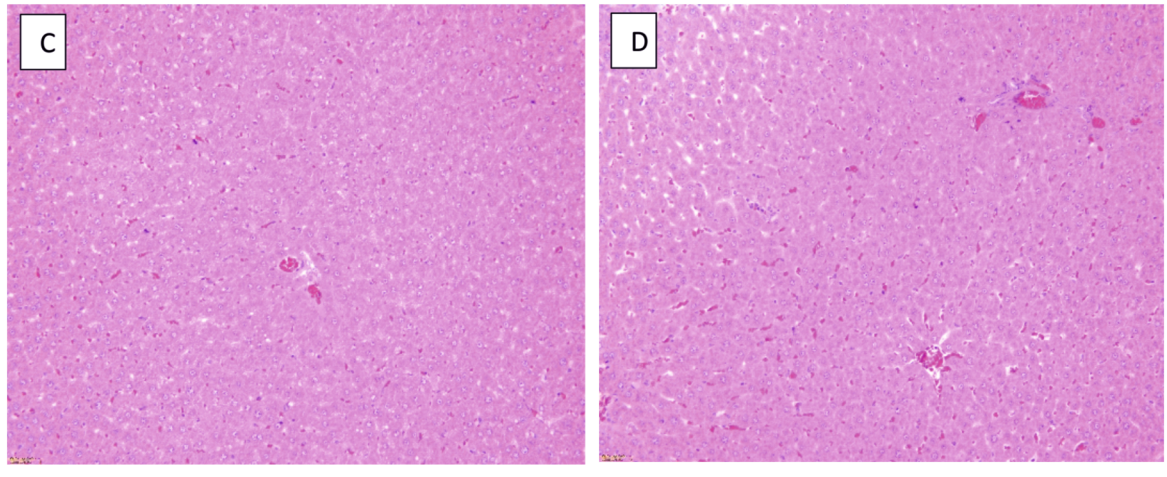

Fig. 8. Histological aspect of the liver C – lot HSZN; D – lot HHZN (HE, ob x 20 µm)

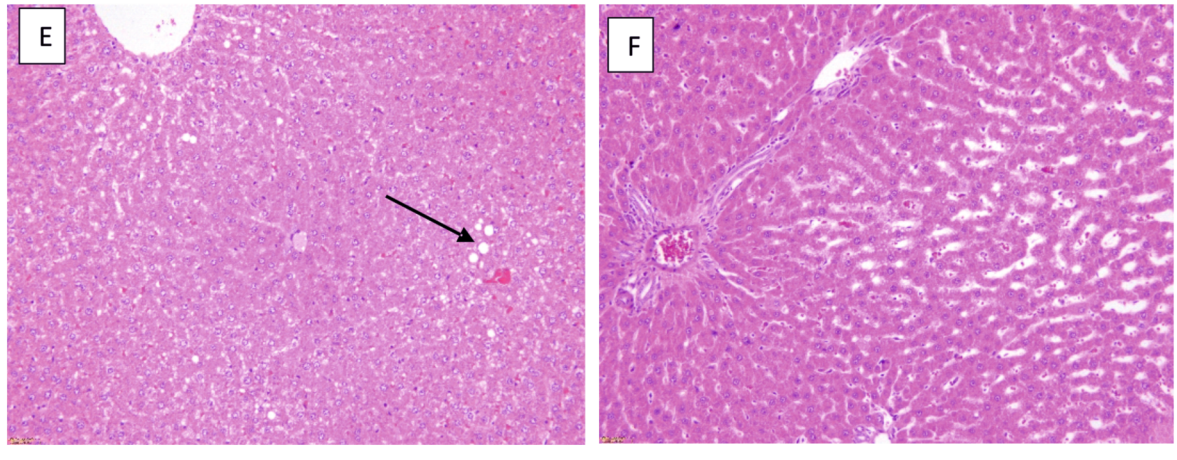

Fig. 9. Histological aspect of the liver: E – lot OB1, mild hepatic steatosis, microvacuolar type; F – lot OB2, (HE, ob x 20 µm)

The most common liver pathologies associated with obesity are steatosis, liver fibrosis, cirrhosis, and less common hepatocellular carcinoma (23).

Regarding the pancreas, the histological picture is normal, without changes, in all the groups studied. No lesions were observed in the acinar epithelial cells or the islets of Langerhans. The results obtained are contradictory to those obtained by Rojas et al., who identified in Sprague-Dawley rats fed a hypercaloric diet a higher incidence of haemorrhage and pigment accumulation in the periphery of the pancreatic islets compared to control groups fed a diet standard. But Imaoka et al. (2007) ob-

tained results similar to ours, noting that feeding geriatric male rats a high-fat/protein diet compared to standard chow produced no changes in pancreatic islets.

Examination of the renal tissue did not reveal the presence of inflammatory infiltrates or renal epithelial cell damage. These microscopic aspects are contradictory to the results obtained by Rojas et al. (2018), where both sexes from the groups that received hypercaloric food presented minimal to moderate renal tubular changes (tubular basophilia and/or tubular dilatation with the presence of hyaline cylinders).

CONCLUSIONS

The study's results suggested that administering hyperlipidic food could induce early insulin resistance (HOMA-IR >1.9) in the MO group compared to the MS group with an optimal HOMA-IR index. The administration of lactoserum Zonar prevented the onset of insulin resistance in all supplemented groups, in both preventive and palliative forms. Considering the results obtained, it can be stated that lactoserum Zonar is neither hepatotoxic nor nephrotoxic, having even a slight antioxidant action at the renal level, and could also decrease organ weight gain in obese rats fed a high-fat diet.

Administration of lactoserum prevented the accumulation of triglycerides in adipocytes, an effect observed in all groups supplemented in preventive and palliative form. The same preventive results are also confirmed by the histomorphometric analysis of the adipocytes, where a decrease in their diameter was found, respectively, an increased number compared to the MO group, where the diameter of the adipocytes was significantly increased and the number of adipocytes decreased. The best results were observed in the groups that received lactoserum throughout the experimental period, followed by those that received lactoserum only for 4 weeks. Following the histopathological examination of the liver and the absence of pathological lesions in all the experimental groups, we can state that lactoserum is a useful supplement in the prevention of obesity and all its side effects.

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