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The influence of entomological preparations on oxidative stress in subacute inflammation

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Abstract

Background: It was found out that the development of oxidative stress in the inflammatory processes is determined by the action of harmful factors, as well as by the activity of leukocytes, macrophages, monocytes with the production of reactive oxygen species. Preparations of entomological origin have revealed antioxidant effect in various pathological processes. Therefore, in the present study, we determine the influence of imuheptin and imupurin on the evolution of oxidative stress parameters during subacute inflammation.
Material and methods: Subacute inflammation was induced in 40 rats. Imupurin, imuheptin and dexamethasone were administered daily for seven days. Malondialdehyde (MDA), total antioxidant activity (TAA) superoxide dismutase (SOD) activity, pro-oxidant antioxidant balance (PAB), native and total thiols in the serum were measured on the 7th day. One-way ANOVA followed by Bonferroni's post-hoc comparisons tests were performed.
Results: Imuheptin produced non-essential reduction of MDA (15.9±2.4 μM/L), native thiol (84.1±18.04 μM/L) level and a tendency to increase SOD (1033.6±171.4 u/c) activity, compared to the control group ($p>0.05$). Imupurine decreased MDA (14.6±2.0 μM/L), total thiol (85.9±14.7 μM/L) and native thiol (78.36±12.4 μM/L), also restored SOD activity (1117.6±103.7 u/c), increased TAA (0.41 ± 0.02 mM/L, $p<0.05$) compared with the control group. PAB was more influenced by imuheptin (325.82 ± 57.82 HK) than imupurin (340.14 ± 37.09 HK).
Conclusions: Imupurine and imuheptin have shown a tendency to reduce the intensity of free-radical generation from membrane lipids and to restore antioxidant capacity.
Key words: imupurin, imuheptin, malondialdehyde, superoxide dismutase, total antioxidant activity, thiol.

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Introduction

Inflammation is a natural defense mechanism against pathogens and it is associated with many pathogenic diseases such, as microbial and viral infections, exposure to allergens, radiation and toxic chemicals, autoimmune and chronic diseases, obesity, consumption of alcohol, tobacco use etc. The acute inflammatory response is started by immune cells enabling secretion of various cytokines and chemokines in order to recruit immune cells, macrophages, neutrophils. Neutrophils are the first to adhere to endothelial cells, and they begin to migrate across the vascular wall and also secrete vasoactive and pro-inflammatory mediators. Most of the early vascular changes observed in acute inflammation are due to inflammatory mediators that are released by inflammatory cells at the site of injury. These mediators, including histamine, platelet-activating factors, bradykinin, and thrombin, increase vascular permeability followed by edema and leukocyte extravasation. Accumulation of activated macrophages at the site of injury is the characteristic feature of inflammatory diseases. There are two distinct populations of macrophages: classically activated macrophages, produce excessive oxidative stress and secrete pro-inflammatory cytokines TNFα, IL-1, and IL-6, which contribute to tissue injury by releasing large amounts of highly reactive cytotoxic oxidants to destroy pathogens and alternatively activated macrophages secreting anti-inflammatory cytokines IL-4, IL-10, and IL-13 which suppress inflammation and help in wound resolution by phagocytizing dead neutrophils and synthesizing molecules that are responsible for tissue remodeling. Inflammatory process in many diseases linked with higher production of reactive oxygen species (ROS) induces oxidative stress and reduces cellular antioxidant capacity. Intensive research into the mechanisms of inflammation in the last decade has described the complicated relationship between oxidative stress and inflammation. ROS are key signaling molecules that play an important role in the progression of inflammatory disorders [1-3]. An enhanced ROS generation by polymorphonuclear neutrophils (PMNs) at the site of inflammation causes endothelial dysfunction and tissue injury. The vascular endothelium plays an important role in passage of macromolecules and inflammatory cells from the blood to tissue. Under the inflammatory conditions, oxidative stress produced by PMNs leads to the opening of inter-endothelial junctions and promotes the migration of inflammatory cells across the endothelial barrier [4]. ROS are generated as by-products of cellular metabolism through the electron transport chain in mitochondria as well as via the cytochrome P450. The other major source, where ROS are not produced

as by-products, are the NADPH oxidases that are present in a variety of cells, especially the phagocytes and endothelial cells, which are central to the genesis of the inflammatory response. Overproduced free radicals react with cell membrane fatty acids and proteins impairing their function permanently. Damage of oxidative stress, such as oxidized proteins, glycated products, and lipid peroxidation results in degeneration of cell membrane and tissue. In addition, free radicals can lead to mutation and DNA damage that can be a predisposing factor for many disorders. Furthermore, free radicals are generally too reactive and have a half-life too short to allow direct measurement in cells, tissues, or body fluids. Because molecular products formed from the reaction of free radicals with biomolecules are generally considered more stable than free radicals themselves, most commonly, free radicals have been tracked by measuring stable metabolite concentrations of their oxidation target products, such as malondialdehyde (MDA) etc. To prevent the damaging effects of oxidants, cells have evolved an array of antioxidant defense systems that function to remove ROS. The antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), peroxiredoxins, and thioredoxins are classified as ROS scavengers. While it is clear that ROS are important to the pathogenesis of inflammation and tissue injury, much remains to be learned about how ROS function physiologically and how they contribute to the mechanism of inflammation and tissue injury [5-7].

Material and methods

Anti-inflammatory activity was evaluated in felt pellet-induced granuloma formation. 11 rats were left as the intact group (no manipulations). The others were divided into four groups (n=10). After shaving the fur, the rats were anesthetized and two 26±1 mg of sterile felt pellets were surgically inserted in the groin region. The right pellet was impregnated with Freund's adjuvant. Imuheptin (500 mg/kg, p.o.), imupurin (500 mg/kg, p.o.), dexamethasone (2.5 mg/kg, intraperitoneally) or saline (0.9% NaCl) solution (intraperitoneally) were administered for 7 consecutive days from the day of the felt pellet implantation. The animals were anesthetized on the 8th day and the pellets were extracted together with the granulation tissue formed, blood was collected for biochemical investigations. The level of malondialdehyde (DAM), the content of native and total thiol, the total antioxidant activity (AAT), the activity of superoxide dismutase (SOD) and pro-oxidant antioxidant balance were determined in serum.

Albino rats (160–250 g) were purchased from the Animal House of *Nicolae Testemitanu* State University of Medicine and Pharmacy. The animals were allowed standard access to food and water. Rats were housed at room temperature under conditions of 12 h of light and 12 h of the dark. The experimental procedures involving rats were approved by the Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy.

The entomological preparations obtained from insects of the order *Lepidoptera*, the genus *Lymantria* at the pupal stage (imupurin) and at the egg and pupae stage (imuheptin) were produced by Arena Group SA, Romania. Dexamethasone was purchased from KRKA d.d., Slovenia.

Oxidative stress assessments were performed in the Biochemistry Scientific Laboratory of *Nicolae Testemițanu* State University of Medicine and Pharmacy.

The level of malondialdehyde (MDA) was dosed according to the method described by Galaktionova L.P. et al. (1998) [8] with amendments [9, 10]. The method is based on the spectrophotometric determination of the trimetinic colored complex formed from the MDA interaction with thiobarbituric acid. The DAM content was calculated based on the molar absorption coefficient $\Sigma=1.56 \cdot 10^5$ mol·cm^{-1} and was expressed in μmol/L blood serum.

The determination of total and native serum thiol was performed according to the method described by Erel O. and Neselioglu D. [11] with modifications [9, 10]. In the first phase the amount of native thiol groups was measured after the addition of formaldehyde. For total thiol assay, disulfide bonds were reduced with NaBH4 to free thiol groups, the unused reductant remnants were completely removed by formaldehyde. Mercaptoethanol solutions were used as calibrators.

The determination of total antioxidant activity (TAA) by the ABTS method was performed according to the method described by Re R., et al. [12] with modifications [9, 10]. Antioxidant capacity was estimated in terms of radical scavenging activity using the pre-formed radical monocation of 2.2-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$). Scavenging of the ABTS radical was monitored by measuring the decrease in absorbance at 734 nm. Trolox® was used for the calibration of the method. Inhibition of absorbance versus Trolox® concentration curve was used to express the plasma antioxidant capacity in Trolox equivalent (μmol Trolox® equiv./l).

Superoxide dismutase (SOD) **activity** was estimated according to the method described by Galaktionova L.P., et al. [8] with modifications [9, 10]. The method is based on inhibition of the nitroblue tetrazolium reduction (NBT) in the system containing phenazine methosulfate and NADH under the action of SOD. The degree of inhibition depends on the activity of the enzyme. The activity of the enzyme is expressed in conventional units. The amount of enzyme required for 50% inhibition of the NBT reduction is taken as the unit of SOD activity. Enzyme activity is related to 1L of blood serum.

The determination of the pro-oxidant-antioxidant balance (PAB) was estimated according to the method described by Alamdari DH. et al. [13] with modifications [9, 10]. The assay is based on 3,3',5,5'-tetramethylbenzidine and its cation, used as a redox indicator participating in two simultaneous reactions. PAB is expressed in arbitrary units calculated based on the standard curve and in Hamidi-Koliakos units (HK) based on the percentage of hydrogen peroxide evaluated in the standard solution.

Statistical analysis. The results were presented as mean

Table 1

The influence of entomological preparations on the parameters of the oxidative and antioxidant system in serum in subacute inflammation

Parameters	DAM, µM/L	TAA, ABTS mM/l Trolox® equiv./l	SOD, u/c	Total thiol, µM/L	Native thiol µM/L	PAB, HK units
1. Intact animals (no manipulations)	15.2±2.2	039±0.01 P_{1-5}<0.05	1064.9±170.1	84.19±9.4	76.12±9.03	303.4±49.57
2. Control saline (0.9% NaCl) solution	16.8±1.3 P_{1-2}>0.05	0.395 ±0.02 P_{1-2}>0.05	999.1±115.9 P_{1-2}>0.05	93.97±12.75 P_{1-2}>0.05	90.13±14.11 P_{1-2}>0.05	348.63± 43.79 P_{1-2}>0.05
3. Dexamethasone 2.5 mg/kg, 7 days	16.1±1.0 P_{1-3}>0.05 P_{2-3}>0.05	0.397 ±0.01 P_{1-3}>0.05 P_{2-3}>0.05	1120.4±123.5 P_{1-3}>0.05 P_{2-3}>0.05	96.5±8.1 P_{1-3}>0.05 P_{2-3}>0.05	82.52±8.05 P_{1-3}>0.05 P_{2-3}>0.05	216.92± 28.54 $P_{3-1,3,4,5}$<0.05
4. Imuheptin 500 mg/kg, 7 days	15.9±2.4 P_{1-4}>0.05 P_{2-4}>0.05 P_{3-4}>0.05	0.39±0.01 P_{1-4}>0,05 P_{2-4}>0,05 P_{3-4}>0,05	1033.6±171.4 P_{1-4}>0.05 P_{2-4}>0.05 P_{3-4}>0.05	94.3±19.2 P_{1-4}>0.05 P_{2-4}>0.05 P_{3-4}>0.05	84.1±18.04 P_{1-4}>0.05 P_{2-4}>0.05 P_{3-4}>0.05	325.82± 57.24 P_{1-4}>0.05 P_{2-4}>0.05 P_{3-4}>0.05
5. Imupurin 500 mg/kg, 7 days	14.6±2.0 P_{1-5}>0.05 P_{2-5}>0.05 P_{3-5}>0.05	0.415± 0.02 P_{1-5}<0.05 P_{2-5}<0.05 P_{3-5}>0.05	1117.6±103.7 P_{1-5}>0.05 P_{2-5}>0.05 P_{3-5}>0.05	85.9±14.7 P_{1-5}>0.05 P_{2-5}>0.05 P_{3-5}>0.05	78.36±12.4 P_{1-5}>0.05 P_{2-5}>0.05 P_{3-5}>0.05	340.14± 37.09 P_{1-5}>0.05 P_{2-5}>0.05 P_{3-5}>0.05

± standard deviation (± SD). Statistical significance of the differences was evaluated using one-way ANOVA with Bonferroni post hoc testing. The difference was considered statistically significant when P<0.05.

Results

The animals from the control group with subchronic inflammation expresed an imbalance between the prooxidant and antioxidant system revealed by increasing the DAM level from 15.2 ± 2.2 µM/L to 16.8 ± 1.3 µM/L (P_{1-2}> 0.05), total thiol from 84.19 ± 9.4 µM/L to 93.97 ± 12.7 µM / L (P_{1-2}> 0.05), native thiol from 76.12 ± 9.03 µM/L to 90.13 ± 14,11 µM/L (P_{1-2}> 0.05) and prooxidant-antioxidant balance (PAB) from 303.4 ± 49.57 HK to 348. 63 ± 43.79 HK (P_{1-2}> 0.05). At the same time, the TAA did not change significantly – 0.39 ± 0.01 mM/L vs. 0.40 ± 0.02 mM/L, P_{1-2}> 0.05, but the SOD activity decreased from 1064.9 ± 170.1 u/c. up to 999.1 ± 115.9 u/c. (P_{1-2}> 0.05). Dexamethasone resulted in an insignificant reduction in DAM, a significant reduction in PAB (216.92 ± 28.54 HK) and a restoration of SOD activity (Table 1). Imuheptin after 7 days of administration decreased the DAM level from 16.8 ± 1.3 µM/L to 15.9 ± 2.4 µM/L (P_{2-4}> 0.05) and PAB from 348.63 ± 43.79 HK to 325.82 ± 57.82 HK (P_{2-4}> 0.05). The entomological preparation contributed to the increase of SOD activity from 999.1 ± 115.9 u/c. up to 1033.6 ± 171.4 c.u. (P_{2-4}> 0.05) and total thiol from 93.97 ± 12.75 µM/L to 94.3 ± 19.2 µM/L (P_{2-4}> 0.05). Imupurin caused a more pronounced decrease in DAM level (14.6 ± 2.0 µM/L) and increased SOD activity (1117.6 ± 103.7 u/c.), but had less influence on PAB (340.14 ± 37.09 HK) compared to imuheptin. The total and native thiol in the serum of rats given imupurine practically did not change compared to the intact group (tab. 1).

Discussion

Inflammatory process, linked with higher production of ROS, induces oxidative stress and reduces cellular antioxidant capacity. This study revealed that dexamethasone had decreased lipid peroxidation and products, such as MDA and restore antioxidant enzyme (SOD) activity. It was shown in previous studies that dexamethasone significantly decreased formation of the inflammatory exudates and weight of granulation tissue, increased the percent of neutrophils, decreased leucocites and lymphocyte number and TNF-alpha, IL-1-beta, IL-6 levels [14].

Glucocorticoids play an important role in regulating the inflammatory and immune response by acting on almost all types of immune cells. Glucocorticoids can regulate the phenotype, survival, and functions of monocytes and macrophages; exhibit anti-apoptotic effects promoting the survival of anti-inflammatory macrophages; improve the phagocytic activity of macrophages; stimulate the clearance of neutrophils; inhibit the release of various pro-inflammatory mediators (cytokines, chemokines etc.) and ROS; can regulate the maturation, survival, and migration toward the lymph nodes and motility of dendritic cells. Corticosteroids inhibit transcription factors that control synthesis of pro-inflammatory mediators, including macrophages, eosi-

nophils, lymphocytes, mast cells, and dendritic cells. Another important effect is inhibition of phospholipase A2, which is responsible for production of multiple inflammatory mediators. Glucocorticoids inhibit genes responsible for expression of cyclooxygenase-2, inducible nitric oxide synthase, and pro-inflammatory cytokines, including tumor necrosis factor alpha and various interleukins. In contrast, corticosteroids initiate upregulation of lipocortin and of annexin A1, a protein that reduces prostaglandin and leukotriene synthesis and that also inhibits cyclooxygenase-2 activity and reduces neutrophil migration to inflammatory sites [15, 16].

Eicosanoids, which control many complex physiological and immunological functions in vertebrates and invertebrates, may be involved in the balance of the prooxidant and antioxidant system of insects. It was studied the influence of eicosanoids inhibitors, such as dexamethasone (0.001%), esculetin (0.001%) and phenidone (0.1%) on oxidative stress parameters (DAM level and glutathione S-transferase (GST) activity) in *Galleria mellonella* larvae on artificial diets containing 0.05% xanthotoxin (XA) for 2 days and supplemented with these inhibitors in concentrations mentioned above. Treating larvae of *G. mellonella* with XA induced lipid peroxidation as evident from the increased content of malondialdehyde (MDA) and antioxidative enzymatic response in a dose-dependent manner. Relative to control, eicosanoid biosynthesis inhibitors (EBIs) – esculetin, dexamethasone and phenidone also resulted in impaired MDA content and antioxidant enzyme activities. MDA and antioxidant enzymes – SOD, GST and glutathione peroxidase (GPx) activities exhibited an incremental increase while catalase (CAT) activity was decreased in the experimental larvae that had been reared on media amended with esculetin, dexamethasone and phenidone and then challenged with standard XA dose. This oxidative stress was associated with elicited antioxidative responses by increasing SOD, GST and GPx and decreasing CAT activities in hemolymph. From these findings it can be deduced that eicosanoids mediate the antioxidant enzymatic responses of insects to food pro-oxidants [17].

Lepidoptera, one of the most widespread and widely recognizable insect orders in the world is characterized by the life cycle of eggs, larvae, pupae, adults with a high content of lipids, proteins, carbohydrates, antioxidants, essential and non-essential amino acids [18-20]. The content at each stage of development depends largely on the feeding of the larvae with the assimilation of vegetal compounds with their subsequent transfer to pupae, adults and eggs. The larvae use the primary metabolites (carbohydrates, lipids, proteins) that participate in nutrition and essential metabolic processes, such as growth, development or reproduction, and the secondary metabolites, such as alkaloids, terpenoids and phenolic compounds. Phenolic compounds consist of flavonoids, tannins and phenolic acids and contain several phenolic rings (polyphenols) with several hydroxyl groups. The digestive tract of larvae ensures the assimilation of nutrients and the transformation of secondary vegetal compounds in various chemical and metabolic processes. During the pupae stage, the larval structures of the insect are slowly broken down, while adult structures (such as wings) are formed. Different types of flavones and flavonol glycosides have been detected in the pupae of butterflies, and these compounds are subsequently transferred to the wings of adult butterflies. Pupae are inactive and many *lepidopterans* produce a cocoon from fibrous protein, i.e., silk, as well as secondary compounds (alkaloids, flavonoids, amino acids, catechins, quercetin, etc.). Flavonoids can protect the cocoon from radiation or increase its antioxidant capacity. *Bombix mori* cocoon extracts have shown antimicrobial activity that is probably due to antimicrobial peptides. Most compounds are also detected in eggs with a protective role against the environment and possible predators [21-24]. Antioxidant defense components protect insects from oxidative stress by scavenging ROS. There were investigated the effects of organophosphorus insecticide, malathion in different concentrations on the activity of SOD and acetylcholinesterase (AChE), glutathione level (GSH) and DAM as biomarkers of oxidative stress in *Galleria mellonella* larvae. The diet with the lowest concentration of malathion (0.01 ppm) did not significantly influence the DAM content and AChE activity, but at 1.0 ppm caused a significant increase in DAM levels, decreased AChE and SOD activity and decreased GSH content [25]. It was examined the effect of long-term exposure to environmentally relevant concentrations of dietary fluoranthene (6.7 and 67 ng / g dry food weight) on defense mechanisms of the *Lymantria dispar*. The activities and expression of isoforms of SOD and CAT, the activities of GST and glutathione reductase (GR), and GSH were determined in the whole midgut and midgut tissue, while SOD and CAT activities were assessed in hemolymph of the larvae. It was shown significantly increased activity of SOD in the whole midgut and midgut tissue, also increased CAT activity in midgut tissue. Significantly decreased SOD activity and increased CAT activity in hemolymph of *L. dispar* larvae were recorded. The tissue-specific responses of enzymes to dietary fluoranthene enabled the larvae to overcome the pollutant induced oxidative stress [26]. CAT, Cu/ZnSOD and Mn-SOD, found in different tissues of *Lymantria dispar* larvae play an important role in the protection against oxidative stress of the environment. When treated with the pesticide avermectin in sublethal doses, a higher expression of CAT and Cu/Zn-SOD was found after 2 hours and of Mn-SOD after 6 hours. The cuticulas transcribed Cu/ZnSOD mRNA and Mn-SOD mRNA significantly higher than other parts of insect body after spraying avermectin of sublethal concentration. The results suggested that CAT and SOD are important antioxidant enzymes for defense against pesticide-induced stress in *Lymantria dispar*, and Cu/ZnSOD isoform has a faster and stronger response [27, 28].

Methanolic extracts from the *Chrysomya albiceps, Lucilia sericata* and *Musca domestica* larvae exhibited a higher antioxidant activity than water extracts, revealed by free radicals scavenging and increasing total antioxidant activity [29].

The products of some insects (bees, wasps, etc.), such as royal jelly, propolis are studied and widely used in traditional medicine, and recent research has shown that they contain a wide range of compounds with multiple pharmacological effects, including antioxidant. Experimental studies have shown that the antioxidant activity of royal jelly is due to increased GSH levels, reduced lipid peroxidation, free radical generation and DAM production, increased concentration of antioxidant enzymes – SOD, CAT, glutathione reductase (GR) and GPx. Royal jelly administration in radiation-induced lung and liver damage reduced oxidative stress and increased antioxidant properties, decreased nitric oxide (NO) and ROS, increased SOD activity and glutathione levels. Hydroxyl radicals and hydrogen-peroxide scavenging activity were verified with 29 antioxidant peptides isolated from RJ hydrolysate, in which 12 small peptides having 2–4 residues (Ala-Lys, Phe-Arg, Ile-Arg, Lys-Phe, Lys-Leu, Lys-Tyr, Arg-Tyr, Tyr-Asp, Tyr-Tyr, Leu-Asn-Arg, and Lys-Asn-Tyr-Pro) had the strongest activity. Moreover, three dipeptides (Lys-Tyr, Arg-Tyr, and Tyr-Tyr) in RJ indicate strong scavenging activity due to a donation of the hydrogen atom from their phenolic hydroxyl group [30].

Phenolic compounds (flavonoids and phenolic acids), substances that express the ability to scavenge free radicals, are mainly responsible for the antioxidant capacity of bee products. Flavonoids are plant derivatives with a polyphenolic structure comprising several subgroups, such as flavones, flavonols, flavanones flavanonols, flavanols (catechins), anthocyanins and chalcones, as well as isoflavones and neoflavonoids. The presence of phenol groups in flavonoid molecules gives them antiradical activity. Phenolic acids are compounds that possess carboxylic groups and phenol, which determine their antioxidant activities, including the prevention of oxidation and generation of oxygen species, as well as the chelation of prooxidant metals. Non-phenolic compounds also can be responsible for the antioxidant capacity of propolis. It was noted antioxidant activity exhibited by hydroxy dicarboxylic fatty acids with 8–12 carbon atoms in the chain and their derivatives. 10-hydroxydecanoic acid, 10-hydroxy-2-decenoic acid and sebacic acid were identified in royal jelly [31].

Terpenes are another class of compounds that have antioxidant action. Triterpenes have been described to act as free radicals scavengers (superoxide anion, hydroxyl radical), also they prevent lipid peroxidation and modify the activity of antioxidant enzymes (SOD, CAT, GPx) [32]. Alpha- and beta-amyrins, non-phenolic compounds found in propolis, belong to triterpenoids and revealed antiapoptotic, antioxidant, anti-inflammatory and antifibrotic, gastro- and hepatoprotective effects [31].

Tenebrio molitor larvae have been consumed worldwide for their nutritional value, which includes high protein, mineral, and unsaturated fatty acids, and were officially registered as a new food ingredient in Korea in 2015 [33, 34]. In previous studies, analyses of their functional properties revealed that *Tenebrio molitor* larvae ethanol (EtOH) extract inhibited the expression of TNF-α and IL-6 in the inflammation-induced RAW 264.7 cell line and had a DPPH radical scavenging ability similar to that of blueberry extract [33, 35].

Sericin is a globular protein from silk fiber, it protects against unfavorable factors and the environment. The antioxidant potential of the sericin is related to its high content of amino acids with hydroxyl groups (mostly serine), which act as chelators [36]. Sericin protein consists of 18 different amino acids, most of them are characterized as polar and the differences observed among the authors regarding the amino acid proportion is due to the method of sericin extraction. Nevertheless, all authors agreed that serine was the most abundant amino acid, followed by aspartic acid and glycine [37]. Among the antioxidant mechanism of the sericin, the inhibition of the tyrosinase activity has been widely studied. Several studies have reported anti-tyrosinase activity of sericin from cocoons belonging to different strains and from different extraction methods as well. The hydrolyzed sericin had increased anti-tyrosinase activity and some authors suggest that the reason behind it is the metal-chelating ability. This effect is due to the presence of a high content of amino acids with hydroxyl groups, such as serine, asparagine and threonine acting as chelators [33, 36]. Likewise, biopeptides of sericin, obtained by enzymatic hydrolysis, exhibited a higher antioxidant potential compared to non-hydrolyzed sericin [37]. It was shown in several studies that active compounds, such as the essential amino acids arginine, histidine, lysine, methionine, cysteine and proteins have an antioxidant capacity, revealed by the ability to reduce iron, scavenging of oxygen and hydroxyl free radicals, as well as hydrogen peroxide scavenging activities. It was concluded that amino acids and proteins inhibit lipid oxidation by biologically designed mechanisms (antioxidant enzymes and iron-binding proteins) or by non-specific mechanisms [38-40].

Conclusions

The analysis of the results of our study allowed us to conclude that Freund's adjuvant produced an inflammatory process with an increase in the level of inflammation mediators, induced an imbalance between the pro- and antioxidant system; dexamethasone diminished the inflammatory process by simultaneously attenuating the formation of ROS and restoring the activity of antioxidant status; imuheptin reduced the level of DAM and increased SOD activity; imupurine decreased the level of DAM and thiol groups with the restoration of SOD and AAT activity. Entomological preparations showed antioxidant activity through their components: unsaturated fatty acids, proteins, peptides and amino acids (serine, arginine, histidine, lysine, methionine, cysteine, etc.); phenolic compounds (flavones, flavonols, flavanones flavanonols, flavanols (catechins), anthocyanins and chalcones, etc.), tannins (proanthocyanids, etc.), alkaloids, terpenoids; antioxidant enzymes (SOD, CAT, GPx, GST). Several mechanisms can be responsible

for antioxidant action of entomological preparations: the presence of hydroxyl groups, which confer the ability to stabilize unpaired electrons; protection of cells against lipid peroxidation; acting as hydrogen donor agents, singlet oxygen and superoxide radicals quencher; activating antioxidant enzymes and metal chelation; free radical scavenging (superoxide anion, hydroxyl radical); changes in the activity of antioxidant enzymes (SOD, CAT, GST, GPx) and the level of endogenous antioxidants (GSH).

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IG designed the study, collected and interpreted the data, drafted the first manuscript.

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Ethics approval and consent to participate
The experimental procedures involving rats were approved by the Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 78 of 22.06.2015).

Conflict of Interests
No competing interests were disclosed.