https://doi.org/10.52645/MJHS.2023.3.03

UDC: 615.324:595.78:616-002-092.9

RESEARCH ARTICLE

The impact of imuheptin and imupurin on cytokine profile and antioxidant status in rat model of inflammation

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ABSTRACT

Introduction. Insects, throughout evolution, have developed a huge arsenal of active compounds, which they use to defend themselves against enemies and diseases, at the same time in recent years insects have shown great interest as a source of food rich in biologically active substances. Research in recent decades has shown that insects produce a variety of proteins and peptides with antibacterial, antifungal, antiviral, immunomodulatory, anti-inflammatory, antioxidant, antitumor, hepatoprotective, antithrombotic, antihypertensive and detoxifying activity during or after contact with the microbial agent or unfavourable factor.

Material and methods. The anti-inflammatory effect of imuheptin and imupurin was investigated in a rat model of subacute inflammation induced by subcutaneous implantation of felt discs. The intensity of the exudative and proliferative phase of inflammation, cytokine profile (TNFalpha, IL-6, IL-10), ceruloplasmin and antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and glutathione-S-transferase) in the serum of rats were evaluated.

Results. Imuheptin and imupurin reduced the level of pro-inflammatory cytokines (TNF-alpha, IL-6) and increased that of the anti-inflammatory cytokine (IL-10), as well as ceruloplasmin, glutathione reductase and glutathione peroxidase in subacute inflammation. Additionally, imupurin significantly increased the level of catalase and imuheptin that of glutathione-S-transferase.

Conclusions. Imuheptin and imupurin determined a moderate effect of inhibiting the exudative and proliferative processes, compared to the reference preparation - dexamethasone, but with a favourable effect on the cytokine profile, decreasing the level of pro-inflammatory cytokines (TNF-alpha, IL-6) and increasing the level the anti-inflammatory one (IL-10), as well as the modulation of antioxidant enzyme activity.

Keywords: imuheptin, imupurin, inflammation, cytokine, antioxidant status.

Cite this article: Gutu I, Bacinschi N, Gudumac V. The impact of imuheptin and imupurin on cytokine profile and antioxidant status in rat model of inflammation. Mold J Health Sci. 2023;10(3):18-24. https://doi.org/10.52645/MJHS.2023.3.03

Manuscript received: 11.07.2023

Accepted for publication: 01.09.2023

Published: 20.09.2023

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

At the moment there is limited data on the anti-inflammatory properties and the mechanism of achieving the anti-inflammatory potential of the entomological preparations – imuheptin and imupurin.

The research hypothesis

Preparations of entomological origin (imuheptin and imupurin) through the content of biologically active substances will improve the evolution of the inflammatory process and restore the imbalance of the pro- and antioxidant systems in rat model of felt-pellet-induced granuloma formation.

The novelty added by manuscript to the already published scientific literature

The ability of imuheptin and imupurin to reduce the level of pro-inflammatory cytokines and increase the level of IL-10 with anti-inflammatory functions, as well as to modulate the activity of antioxidant enzymes in subacute inflammation was revealed.

Introduction

The current arsenal of preparations for the treatment of inflammatory processes consists of non-steroidal, steroidal, and disease-modifying antirheumatic drugs (DMARDs) with good efficacy, but safety issues require the research of new substances with anti-inflammatory properties, possibly with different mechanisms and increased safety profiles. Currently, there is a varied and documented basis of methodological recommendations for the *in vitro* and *in vivo* study of the anti-inflammatory properties of new substances that allow determining the influence of the investigated substances on inflammatory processes with the elucidation of the mechanisms and peculiarities of action [1-4].

Insects have become an object of research due to their ability to survive in adverse environmental conditions, including infectious factors and those that produce inflammatory processes. Bioactive compounds such as phenols, flavonoids, terpenes, saponins, sugars, alkaloids, glycosides and fatty acids, identified in a wide variety of insects, have demonstrated biological properties including antioxidant, anti-inflammatory, antiproliferative, cytotoxic, analgesic, immunomodulatory, antidiabetic, cardioprotective, antihypertensive, antimicrobial properties. Analysis of literature data demonstrated that a number of extracts, peptides, and synthetic analogues exhibite anti-inflammatory properties [5-8].

Previous preclinical and clinical studies of preparations of entomological origin obtained from *Lymantria dispar* at different stages of development (entoheptin, imuheptin, imupurin, adenoprosine) have shown hepatoprotective, immunomodulatory, anti-inflammatory properties [9, 10]. The purpose of the study was to determine the influence of preparations of entomological origin (imuheptin and imupurin) on the exudative and proliferative processes of subacute inflammation.

Materials and methods

This experimental study was conducted in the Department of Pharmacology and Clinical Pharmacology and the Biochemistry Scientific Laboratory of *Nicolae Testemiţanu* State University of Medicine and Pharmacy. Albino rats were purchased from the Animal House of *Nicolae Testemiţanu* 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 dark. The experimental procedures involving rats were approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, Minutes No. 78 from 22.06.2015. 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.

Adult male Wistar rats (180-330 g) were used for the study. They were randomly divided into the following groups: intact (n=8) – no manipulations, only saline (0.9% NaCl) solution intraperitoneally was administered; control (n=6) – felt pellets were implanted, saline (0.9% NaCl) solution intraperitoneally was administered; standard (n=9) - felt pellets were implanted, the steroid anti-inflammatory drug dexamethasone was administered; treatment 1 (n=7) - imuheptin was administered; treatment 2 (n=9) – imupurin was administered. In all animals, except intact group, subacute inflammation was induced by implanting felt pellets, weighing 26±1 mg, in the groin region of the animal's body on the right and left sides (1st day). The intervention was performed in aseptic conditions, under general anaesthesia with sodium thiopental (50 mg/kg intraperitoneally). Substances of entomological origin (imuheptin, imupurin) were administered daily internally for seven days, in doses of 500 mg/kg, dexamethasone (the reference preparation) – in a dose of 2.5 mg/kg intraperitoneally. On the 8th day, under general anaesthesia, the pellets were extracted together with the formed granulation tissue, weighed wet, and then dried at 60°C to constant weight.

The degree of the exudative reaction was assessed calculating the difference between the weight of the wet and the dry granuloma, and the percentage of inhibition of the exudative phase. Proliferative reaction was evaluated calculating the difference between the weight of the dry granuloma formed and the initial weight of the pellet, as well as the percentage of inhibition of the proliferative phase. To calculate the percentage of inhibition of the exudative and proliferative phases, the formula was used:

$$Pi = \left(1 - \frac{Mt}{Mm}\right) * 100$$

where: *Pi* – the percentage of inhibition;
Mt – wet/dry weight of granuloma in treated group;
Mm – wet/dry weight of granuloma in control group [1, 3].

The level of TNF-α, IL-6 and IL-10 was determined in the serum of rats by the ELISA method, using Invitrogen kits, ThermoFisher Scientific Inc, USA. The activity of catalase, superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPO), glutathione-S-transferase (GST) and ceruloplasmin (CP) was determined according to the methods described by Gudumac V. *et al.* [11, 12].

***Statistical analysis*:** The results were statistically processed using the functions of the computer program SPSS (version 25.0) and the basic indicators of descriptive statistics were determined – mean and standard deviation. The differences between the groups were analyzed using One –Way ANOVA, followed by *post hoc* Bonferonni test. The significance threshold set was for the 95% confidence interval.

Results

The data presented in table 1 showed that the initial weight of subcutaneously implanted felt pellets was almost identical in all groups. After extracting the pellets with the granuloma formed around them, it was found that their weight increased significantly in all groups, which proves the development of the inflammatory process. Thus, the wet granuloma weight in the control group was 315.1±32.0 mg (increased 11.2 times), in the dexamethasone group 196.1±10.0 mg (increased 7 times), in the imuheptin group 273.1±24.2 mg (increased 9.7 times), and with imupurin 267.5±34.4 mg (increased 9.5 times). Thus, we can state that the implantation of the felt pellets caused a marked exudative inflammatory reaction. In order to assess the influence of the investigated preparations on the exudative phase, the percentage of inhibition was calculated (tab. 1). Dexamethasone caused an inhibition of the exudative process by 38%, imuheptin by 13%, and imupurin by 15%. The analysis of the weight of the dry granuloma revealed that in the control group, it was 90.4±12.0 mg or 3.2 times higher than the initial weight of implanted pellet, which reveals the presence of a marked proliferative process. In the group treated with dexamethasone, the dry granuloma weight was 49.9±6.1mg or 1.8 times higher than the initial weight, but significantly reduced compared to the control group. In the group treated with imuheptin, the weight of the dry granuloma was 73.4±10.7 mg or 2.6 times higher than the initial one, and in the group with imupurin 73.0±16.7 mg or 2.6 times higher. The intensity of the proliferative process was analyzed, calculating the percentage of inhibition, which for dexamethasone was 45%, for imuheptin 19% and for imupurin 20%. These data confirm that dexamethasone essentially reduced the proliferative inflammation, and preparations of entomological origin showed a moderate effect. Based on the results obtained, we can conclude that dexamethasone effectively inhibited the exudative and proliferative phases in subacute inflammation, and the entomological preparations mainly decreased the proliferative phase.

Table 1. The effects of imuheptin and imupurin on the exudative and proliferative phase of subacute inflammation in rats

Treatment	Initial weight of pellets	Wet weight	The percentage of inhibition of exudative phase	Dry weight	The percentage of inhibition of proliferative
Control, saline solution	28.1±0.4	315.1±32.0		90.4±12.0	
Dexamethasone 2.5mg/kg	28.1±0.9	196.1±10.0*	38%	49.9±6.4*	45%
Imuheptin 500 mg/kg	28.1±1.0	273.1±24.2	13%	73.4±10.7	19%
Imupurin 500 mg/kg	28.2±0.9	267.5±34.4	15%	73.0±16.7	20%

Note: Values expressed as mean ± SD, SD – standard deviation; the results were analyzed using Oneway ANOVA followed by Bonferroni multiple comparison test; *- P < 0.05 was used to indicate statistical significance when compared to control

At the same time, in the control group with subacute inflammation, the level of TNF-alpha increased compared to the intact group (48.68±10.77 pg/ml) and constituted - 72.67±20.19 pg/ml (P1-2<0.05); increased IL-6 level was observed - 37.57±1.69 pg/ml (P1-2<0.05) compared to the control group (33.75±0.57 pg/ml); as well as the decrease in IL-10 content - 15.28±2.36 pg/ml (P1-2<0.05) compared to the control group, where the level was 32.35±13.39 pg/ml (table 2). Dexamethasone caused a significant decrease in TNF-alpha level and IL-6 level, also slightly increased the IL-10 level. Preparations of entomological origin decreased the level of TNF-alpha and that of IL-6. Imuheptin, and especially imupurin, increased the content of IL-10, a cytokine with anti-inflammatory properties, compared to the control group (table 2). Thus, the steroid anti-inflammatory mainly decreased the level of pro-inflammatory cytokines (TNF-alpha, IL-6), and preparations of entomological origin restored the ratio between pro-inflammatory (TNF-alpha, IL-6) and anti-inflammatory (IL-10) cytokines.

Table 2. The influence of imuheptin and imupurin on cytokines and ceruloplasmin level in rats serum with felt-pellets induced granuloma

Treatment	TNF - alpha, pg/ml	IL-6, pg /ml	IL-10, pg /ml	CP, mg/L
Intact (no pellets were implanted)	48.7±10.8	33.7±0.6	32.3±13.4	470.4±87.0
Control, saline solution	72.7±20.2 §§	37.6±1.7§§	15.3±2.4 §§	390.7±78.9
Dexamethasone 2.5 mg/kg	43.3±6.5*	34.2±1.4*	21.2±5.6§§	323.8±42.5§§
Imuheptin 500 mg/kg	46.1±10.9*	35.1±1.5*	23.3±6.6	539.7±68.5*/**
Imupurin 500 mg/kg	46.2±12.9*	34.1±1.0*	27.4±4.2*	501.5±66.4*/**

Note: Values expressed as mean±SD, SD – standard deviation; the results were analyzed using One Way ANOVA followed by Bonferroni multiple comparison test; TNF alpha - tumour necrosis factor alpha ; IL – interleukin; CP - ceruloplasmin; §§ - P<0.05 was used to indicate statistical significance when compared to intact group; * - P<0.05 was used to indicate statistical significance when compared to control group; ** - P<0.05 was used to indicate statistical significance when compared to dexamethasone group.

In felt-pellets-induced granuloma, a decrease in the level of ceruloplasmin was found - from 470.41±87.0 in the intact group to 390.68±78.96 mg/L (P>0.05) in the control group. Dexamethasone caused an even more pronounced reduction in ceruloplasmin levels. Imuheptin and imupurin significantly increased the content of ceruloplasmin compared to the control group with subacute inflammation (table 2). Withal, a tendency to decrease the activity of catalase, SOD and GPO and to increase GR was found in the control group, without essential changes in GST. Dexamethasone virtually restored the activity of catalase and SOD, the level of these enzymes being comparable to that of the intact group, and increased the activity of enzymes of the glutathione system (GR, GPO, GST). Imuheptin, administered to animals with inflammation, reduced SOD activity and restored catalase activity compared with the control group, and increased GR, GPO and GST activity. Compared to the control group, imupurin increased the activity of catalase and decreased that of SOD and GST, simultaneously increasing GR and GPO levels (table 3).

Discussion

The screening of the anti-inflammatory properties in the previous research, namely formaldehyde-induced paw oedema allowed us to find that the drugs of entomological origin (entoheptin, imuheptin, imupurin) do not prevent inflammation but had an anti-inflammatory activity comparable to that of diclofenac. The comparative analysis between the anti-inflammatory potential of entoheptin, imuheptin, imupurin and diclofenac revealed that entoheptin possesses the strongest anti-inflammatory activity, achieving complete healing in 48 hours, followed by diclofenac and imuheptin. Imupurin showed the weakest anti-inflammatory action, but it was more intense than in the group of untreated animals [9, 10].

Table 3. The influence of imuheptin and imupurin on antioxidant enzymes in rats serum with felt-pellets induced granuloma

Treatment	Catalase μM/s.L	SOD c/u	GR, nM/s.L	GPO, nM/s.L	GST, nM/s.L
Intact (no pellets were implanted)	19.7±1.7	918.1±45.7	64.8±18.9	430.8±90.3	24.5±12.9
Control, saline solution	15.9±3.2	905.3±49.2	80.4±21.9	380.3±42.3	24.8±10.3
Dexamethasone 2.5 mg/kg	18.3±2.4	944.9±79.3	99.2±33.5	531.7±116.8	50.2±13.4$^{§§/*}$
Imuheptin 500 mg/kg	20.4±2.9	865.0±96.6	127.6±21.7§§	551.8±96.8*	34.3±8.2
Imupurin 500 mg/kg	31.8±9.5$^{§§/*/**}$	888.9±135.9	150.8±65.7$^{§§/*}$	535.5±100.6*	21.1±9.5**

Note: Values expressed as mean±SD, SD – standard deviation; the results were analyzed using One Way ANOVA followed by Bonferroni multiple comparison test; SOD - superoxide dismutase; GR - glutathione reductase; GPO - glutathione peroxidase; GST - glutathione-S-transferase; §§ - P<0.05 was used to indicate statistical significance when compared to intact group; *** -** P<0.05 was used to indicate statistical significance when compared to control group; **** -** P<0.05 was used to indicate statistical significance when compared to dexamethasone group.

Insects include the largest number of species and play an important role in the terrestrial ecosystem and have been considered a useful natural resource as food, especially due to their protein and fatty acids. Some studies have shown that insects not only have a high protein content, but micronutrients and bioactive peptides with various pharmacological effects, including anti-inflammatory, antioxidant, antimicrobial and antitumor activity. Wasps (*Vespa orientalis*) have a major protein content, and the aqueous extract of *Vespa affinis* has demonstrated antioxidant effects by activating the antioxidant enzymes glutathione-S-transferase (GST) and catalase (CAT) [13-16].

Oxidative and inflammatory processes are closely related, so antioxidants annihilate free radicals that damage cells and lead to inflammation. Several studies have shown that antioxidant and anti-inflammatory peptides have protective effects against reactive oxygen species (ROS) and can contribute to a significant reduction in oxidative stress levels. *Tenebrio molitor*, *Schistocerca gregaria* and *Grylodes sigillatus* have been shown to be a rich source of bioactive peptides with antioxidant and anti-inflammatory properties, which have shown high antiradical activity and an ability to chelate iron ions and inhibit the activity of lipoxygenase and cyclooxygenase-2 [16, 17].

Subacute and chronic inflammation is a response to prolonged stimulation of proinflammatory factors on tissues and is characterized by leukocyte infiltration at the site of inflammation, fibrosis, and granuloma formation. The mechanism of chronic inflammation is attributed, in part, to the release of ROS from activated neutrophils and macrophages, excessive cytokine production, dysregulation of cell signalling, and loss of barrier function. This overproduction causes peroxidation of membrane lipids, which leads to tissue damage by damaging macromolecules. ROS cause or extend inflammation by stimulating the release of cytokines (IL-1beta, TNF-alpha, INF-alpha), which stimulate the recruitment of additional neutrophils and macrophages [18].

Subcutaneous implantation of felt pellets causes the formation of granulomatous tissue. This granulomatous tissue is due to the accumulation of macrophages, neutrophils and lymphocytes around the foreign particles, followed by the proliferation of fibroblast cells. The implanted felt pellets stimulate the immune system to produce interleukins and antibodies that stimulate the proliferation of lymphocytes and the accumulation of cells around them. Initially, exudative processes develop through the transudation of liquid and a marked increase in the weight of wet felt pellets. Steroidal and nonsteroidal anti-inflammatory drugs are shown to reduce granuloma size and transudate by inhibiting the production of proinflammatory mediators (inflammatory cytokines, leukotrienes, and prostaglandins), inhibiting cell (leukocyte) infiltration, and preventing fibroblast proliferation and collagen fibre production and mucopolysaccharide synthesis. A similar effect was demonstrated by dexameth-

asone in our study. Imupurin showed a lower ability, compared to dexamethasone, to reduce the exudative and proliferative processes. Possibly, unlike the steroid anti-inflammatory, the preparation of entomological origin develops a slower effect due to its immunotropic properties on cellular immunity - modulation of T-lymphocytes [18, 19].

Implantation of felt discs causes an exudative and proliferative reaction and an increase in the level of pro-inflammatory cytokines TNF-alpha, IL-1beta and IL-6, products that characterize the function of macrophages (activation, infiltration). The administration of indomethacin, a non-steroidal anti-inflammatory, causes a decrease in the mass of the granuloma and the level of IL-6, with an increase in TNF-alpha, without changing that of IL-1beta [20].

When foreign bodies, such as the implantation of felt discs, penetrate the skin, the production of nitric oxide occurs under the action of nitric oxide synthase. Subsequently, the cascade of proinflammatory mediators and cytokines is activated which includes cyclooxygenase 2, interleukins IL-1β and IL-6, and TNF-alpha. These pro-inflammatory mediators and cytokines cause the activation of the classical inflammatory pathway, nuclear factor NF-κB and mitogen-activated protein kinase (MAPK) triggering an uncontrolled inflammatory response. The use of wasp venom suppressed the production of nitric oxide and reduced the mRNA expression of IL-1β, IL-6 and TNF-α [21].

Glucocorticoids (GCs) play an important role in the regulation of the inflammatory and immune response, acting on most types of immune cells. Glucocorticoids can: regulate the phenotype, survival and functions of monocytes and macrophages; exhibits anti-apoptotic effects that promote the survival of anti-inflammatory macrophages; improve the phagocytic activity of macrophages; stimulates the clearance of neutrophils; inhibits the release of pro-inflammatory mediators (cytokines, chemokines, etc.) and reactive oxygen species; regulate the maturation, survival and migration to lymph nodes and the functionality of dendritic cells. Glucocorticoids inhibit transcription factors that control the synthesis of proinflammatory mediators and cells, including macrophages, eosinophils, lymphocytes, mast cells, and dendritic cells. Another important effect is the inhibition of phospholipase A2, responsible for the production of pro-inflammatory mediators. Glucocorticoids inhibit the genes responsible for the expression of cyclooxygenase-2, iNOS and proinflammatory cytokines. Concomitantly, GCs produce an increase in lipocortin and annexin A1, with subsequent reduction in the synthesis of prostaglandins and leukotrienes [22-24].

The plasma level of ceruloplasmin, considered an acute-phase inflammatory plasma protein, produced predominantly by hepatocytes and activated monocytes and macrophages, increases in response to inflammation, trauma, or infection. Ceruloplasmin production by myeloid cells is induced by interferon-γ (IFN-γ) and tumour necrosis factor-alpha (TNFα). The ferroxidase activity of ceruloplasmin inhibits the ferrous ion-mediated production of reactive oxygen species with the manifestation of antioxidant activity. Ceruloplasmin also exhibits ferroxidase-dependent bactericidal activity. The increase in the plasma level of ceruloplasmin during the acute phase reaction suggests a possible anti-inflammatory function of the antioxidant, bactericidal and ferroxidase activity. Ceruloplasmin, due to its antioxidant activity, prevents the carbonylation of proteins by reactive oxygen species in inflammatory diseases. The anti-inflammatory action of ceruloplasmin, most likely, is determined by its synthesis by infiltrated macrophages at the site of inflammation and, less so, by the modulation of the T-cell response. Thus, the prevention of oxidation and tissue damage can be considered the basic mechanism of ceruloplasmin, generated by macrophages recruited to the site of inflammation [25].

Most organisms use aerobic cellular respiration to produce energy for their functioning, but this process is also accompanied by side effects caused by metabolic products in the form of free radicals. Living organisms use exogenous and endogenous antioxidants to defend themselves against the harmful effects of free radicals, and studies on the antioxidant activity of substances of plant, animal, entomological, or biological origin have captured the interest of researchers for many years. [26]. Antioxidants are compounds capable of counteracting the effects of oxidative processes in cells or exogenous systems, reacting in particular with reactive oxygen or nitrogen species or with other free radicals or unstable molecules generated during normal metabolic oxidative reactions. Antioxidant systems include enzymes (SOD, catalase, GP, GR, GST) and non-enzymatic substrates (glutathione, coenzyme Q, ascorbic acid, retinols, tocopherols, flavonoids, carotenoids, etc.). Antioxidants are found in products of vegetable, animal, or entomological origin, in food supplements. Antioxidant capacity is the general ability of organisms or compounds to interact with free radicals and prevent their harmful effect. The antioxidant effect includes the protection of cells and cellular structures against the effect of free radicals, especially oxygen and nitrogen [26-28].

The enzymes of glutathione metabolism - glutathione reductase (GR), glutathione peroxidase (GPO) and glutathione-S-transferase (GST) constitute a group of antioxidants that ensure the protection of cells against ROS and RNS action, also against lipid peroxidation products. The main role in the degradation of hydroperoxides belongs to the GPO/GR enzyme system. GR has a variable distribution in organs and intracellular organelles and ensures the maintenance of the optimal level of glutathione (GSH) by reducing oxidized GSH (GSSG). The enzyme reduces the need for the new synthesis of GSH from amino acids. GR function is in constant correlation with GPO and GST, enzymes that oxidize GSH in peroxide reduction processes [29, 30].

Glutathione peroxidase catalyzes the cleavage of hydrogen peroxide and organic peroxides by using GSH and converting it to GSSG. This enzyme is in competitive relations, due to its different intracellular localization, with catalase and SOD in the neutralization of excess hydrogen peroxide and organic peroxides, which ensures the efficient functioning of these enzymes. Glutathione peroxidase in mitochondria and peroxisomes works in tandem with catalase, and in the cytoplasm with SOD, which ensures, together with

non-enzymatic antioxidants, the protection of subcellular structures and the modulation of the oxygen activation process by deregulating the formation of the hydroxyl radical (OH•). Glutathione-S-transferase catalyzes the conjugation of GSH with electrophilic organic compounds, an important detoxification reaction of exogenous products and the neutralization of endogenous substances within the physiological processes of metabolic waste elimination [29, 30].

Insect antioxidant systems are of crucial importance in defence mechanisms against xenobiotics that produce endogenous reactive oxygen species (ROS) in insects. Increased levels of radicals from xenobiotics, such as plant secondary metabolites, are associated with oxidative stress in the midgut tissues of lepidopteran larvae. Xenobiotics (prooxidant substances, heavy metals, pesticides) and their metabolism are associated with the production of free radicals, which react with various biomolecules and affect cellular functions. These radicals are removed by innate antioxidant defence systems, including antioxidant enzymes and various antioxidant compounds. Deficiency of the antioxidant defence system leads to increased ROS, which interacts with many cellular biomolecules, including proteins, lipids, enzymes, carbohydrates, and DNA with their damage. Insects, in order to overcome the toxic effects of SRO, have developed a complex antioxidant mechanism consisting mainly of the enzymatic action of glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase, and glutathione transferases (GST). In insects, GSTs are involved in the transformation of many insecticides, and their overexpression is responsible for the development of resistance against those insecticides. Glutathione-S-transferases present selenium-independent glutathione peroxidase activity and can remove highly reactive electrophilic components, lipid hydroperoxides (DAM, trans-4-hydroxy-2-nonenal), generated by ROS-initiated lipid peroxidation. After exposure to xenobiotics, increased levels of DAM have been correlated with a variety of tissue and cell membrane damage in animals [15, 31, 32].

The anti-inflammatory properties of edible insects have been evaluated *in vivo* and in cellular models. *In vivo*, studies have revealed a reduction in circulating cytokine levels elevated by various stressors after administration of different insect extracts. An increase in cytokine levels was found only at high doses of *Hermetia illucens* administered to healthy fish, without being confirmed by inflammatory events on histological analysis. In studies of healthy subjects, circulating levels of TNF-α have been shown to be reduced, data that must be reviewed because they may have had a reduced level of inflammation [32]. Levels of NF-κB, the transcription factor regulatory genes involved in inflammatory responses, were decreased in cell and animal models. At the same time, the levels of TLR4, whose stimulation leads to the activation of NF-kB, were not affected. Some studies have shown activity in reducing the production of NO in macrophages, a radical involved in the modulation of inflammation and immunity. In conclusion, evidence from cellular and animal models supports an effect on reducing inflammatory cytokines by modulating NF-kB levels, without affecting immunoglobulins [33, 34].

Conclusions

Imuheptin and imupurin showed a moderate inhibitory effect, predominantly of proliferative processes compared to dexamethasone, which essentially diminished inflammation's exudative and proliferative phases. Imuheptin and imupurin reduced the level of pro-inflammatory cytokines (TNF-alpha, IL-6) and increased that of anti-inflammatory cytokines (IL-10). The studied entomological preparations increased the ceruloplasmin level and restored the activity of catalase and glutathione peroxidase with the increase of glutathione reductase activity in subacute inflammation. Due to the effects mentioned earlier, the researched entomological preparations – imupurin and imuheptin have an anti-inflammatory potential, which requires a more in-depth study to determine the mechanisms of anti-inflammatory action and the pathological conditions where these effects would be beneficial.

Competing interests

None declared.

Authors' contribution

IG conceived and participated in the study design, performed the experiments and statistical analysis, and drafted the manuscript. NB participated in the study design and helped drafted the manuscript. VG had contribution to acquisition and interpretation of data, and helped drafted the manuscript. All the authors reviewed the work critically and approved the final version of the manuscript.

Ethical Statement

This study was carried out in accordance with the *European Convention for the Protection of Vertabrate Animals Used for Experimental and Other Scientific Purposes* and approved by the Research Ethics Committee of *Nicolae Testemiţanu* State University of Medicine and Pharmacy, Minutes No. 78 from 22.06.2015.

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