

***Nigella sativa* itself and as a part of a balm inhibits activity of purified 20S proteasome**

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Abstract

Background.

Ubiquitin-dependent proteasomal proteolysis is the perspective target for the therapy of the high amount of different diseases. the special attention is paid to the therapy of inflammatory processes of both origins: infection and aseptic. Besides proteolytic processing of the inhibitor of NFkappaB proteasome is important for activation of polymorphonuclear neutrophils (PMN) and neutrophil extracellular traps (NETs) formation. Basing on the mentioned above information we have evaluated some plant extracts included in the medical compound «Healthy Tonsils» that is used for the treatment of the chronic tonsillitis for their ability to inhibit activity of 20S proteasome.

Methods.

For *in vitro* experiments the purified 20S proteasome was used. 20S proteasome was incubated for 30 minutes at 36 C degrees in a mixture with phytobalm components. After the next 30 minutes of incubation with 6 microM solution of corresponded fluorogenic substrates the measurement of fluorescence products was performed (exciting/emission wave length was 360/440) on the spectrofluorimeter Hitachi-4000 with the use of free 7-amido-4-methylcoumarine for a standard curve.

Results.

In the same concentrations the compounds investigated has demonstrated different ability to inhibit activity of purified 20S proteasome. The most effective compound was the extract of *Nigella sativa* that inhibits chymotrypsin-like and caspase-like activities of purified 20S proteasome on 69 % and 85 % correspondingly.

Conclusions.

Thus, our results allow to explain the anti-inflammatory activity of phytobalm particularly by ability of some of its compounds to inhibit proteasomal proteolysis.

Background

Ubiquitin-dependent proteasomal proteolysis is the perspective target for the therapy of the high amount of different diseases. The clinical study in this direction has started from the usage of specific proteasomal inhibitors in the therapy of multiple myeloma and some other cancers [1]. The range of pathologies that possibly can be treated with proteasomal inhibitors was expanded during last years. In particular, the special attention is paid to the therapy of inflammatory processes of both origins: infection and aseptic [2, 3]. This is completely justified by the mechanism of activation of NFkappaB – the

transcription factor under the control of which is the expression of the majority of proinflammatory genes starting from the cytokines, cell adhesion molecules, and finishing with the components of inflammasome [4]. Besides proteolytic processing of the inhibitor of NFκB proteasome is important for activation of polymorphonuclear neutrophils (PMN) and neutrophil extracellular traps (NETs) formation. Proteasomal inhibitors effectively counteract this important mechanism of alteration. The ability of proteasomal inhibitors to induce apoptotic and autophagic cell death mechanisms of inflammatory cells should be also mentioned. Disturbances of these mechanisms may lead to the transition of an inflammation to a chronic state.

The search of proteasomal inhibitors of natural origin has started at the end of XX century and is still going on [5]. Researchers are interested in such molecules due to their low toxicity (debugging of utilization processes in the organism), their high availability, low price, and wide range of action points comparing to the target inhibitors of synthetic origin [6, 7].

Basing on the mentioned above information we have evaluated some plant extracts for their ability to inhibit activity of 20S proteasome. Anti-inflammatory properties of these extracts are already well known in phytotherapy, and this was the main selection criterion for including them in medical compound «Healthy Tonsils» that is used for the treatment of the chronic tonsillitis.

Methods

Plant extracts that compose phytobalm «Healthytonsils»:

- Peru balsam derived from a tree *Myroxylon balsamum* var. *pereirae*
- Viniline
- fir-needle
- *Styphnolóbium japonicum*
- *Melaleuca*
- *Achilléa millefólium*
- *Agrimonia eupatoria*
- *Juniperus communis L.*
- *Nigella sativa*
- *Verbáscum thápsus*

These compounds were soluble in DMSO, and were investigated in a form of solutions in concentrations that correspond to the phytobalm «Healthytonsils».

Measurement of the proteasomal activities For *in vitro* experiments the purified 20S proteasome (ICN, USA) was used. 20S proteasome (2.5 microg) was incubated for 30 minutes at 36 C degrees in a mixture with phytobalm components. After the next 30 minutes of incubation with 6 microM solution of corresponded fluorogenic substrates the measurement of fluorescence products was performed (exciting/emission wave length was 360/440) on the spectrofluorimeter Hitachi-4000 with the use of free 7-amido-4-methylcoumarine for a standard curve. In the each measurement the percentage of the inhibition of correspondent substrate cleavage was estimated under the influence of specific proteasomal inhibitor clasto-lactacystin beta-lactone in probes with the same concentration of DMSO or ethanol.

Statistical analysis. The data analysis was performed using the R statistical environment (version 3.5). All of the quantitative factors were checked for normality of the data distribution when using the Kolmogorov-Smirnov test. One-Way Analysis of Variance (ANOVA) was used to determine the differences between the group averages. The results were considered statistically significant at $p < 0.05$.

Results

Results of the experiments are noted in Table 1. In the same concentrations the compounds investigated has demonstrated different ability to inhibit activity of purified 20S proteasome. The most effective compound was the extract of *Nigella sativa* and Peru balsam derived from a tree *Myroxylon balsamum var. pereirae*. But only the first one of the mentioned compounds demonstrated dose dependent effect (Fig. 1).

Table 1

Percentage of chymotrypsin-like and caspase-like 20S proteasomal activities change under the influence of different components of phytobalm "Healthy Tonsils" and specific proteasomal inhibitor clasto-lactacystin beta-lactone, %.

Substance	Chymotrypsin-like activity	Caspase-like activity
Myroxylon peruferum	-75,9 ± 3,8 %	-44 ± 2,2 %
Vinilinum	+ 93 ± 4,65 %	+ 7,2 ± 0,36 %
fir-needle	-3,8 ± 0,2 %	0 ± 0,007 %
Styphnolobium	+ 19 ± 0,95 %	-3,3 ± 0,17 %
Melaleuca alternifolia	-4 ± 0,2 %	-1,9 ± 0,1 %
Achilléa millefólium	+ 5,8 ± 0,3 %	-2,6 ± 0,13 %
Agrimonia eupatoria	-12 ± 0,6 %	-7 ± 0,35 %
Juniperus communis	+ 0,8 ± 0,04 %	-4 ± 0,2 %
Nigella sativa	-69 ± 3,45 %	-85 ± 4,25 %
Verbáscum	+ 1 ± 0,05 %	0 ± 0,01 %
clasto-lactacystin beta-lactone	-83 ± 4,15 %	-73 ± 3,65 %

Discussion

Thus, as a result of our experiments the ability of *Nigella sativa* extract to influence on the activity of purified 20S proteasome was demonstrated. The study of this substance is very actual due to in particular thymoquinone, the active component of the *Nigella sativa* oil has a very pronounced antiproliferative property demonstrated on different cell lines [9]. Further research in this direction has demonstrated that antiproliferative effects of thymoquinone are in a part caused by the ability to inhibit proteasomal proteolysis [8]. These results support our data due to thymoquinone is one of the compounds of *Nigella sativa* extract. But it is also not excluded that other active components of this extract [10, 11, 12] also have ability to inhibit activity of proteasome. Our results indirectly indicate this suggestion due to the mixture of all components of the balm salved in DMSO also inhibits purified 20S proteasome activity in a dose dependent manner (Fig. 1. C,D). It is not excluded of course that this effect can be explained by the influence of some other components of the balm that have not demonstrated such a property in a single experiment. Clinical observations of the usage of the phytobalm in a children and adolescence suffering from tonsillitis indicate significant decrease of inflammation that was supported also with ultrasound study results.

Conclusion

Thus, our results allow to explain the anti-inflammatory activity of phytobalm particularly by ability of some of its compounds to inhibit proteasomal proteolysis.

Declarations

Ethics approval and consent to participate: The work is performed only *in vitro* without using of cell lines, isolated cells, laboratory animals and patients materials.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The originality of figure(s), table(s). The table and figure that are presented in this manuscript are originally done by authors for this publication.

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Figures

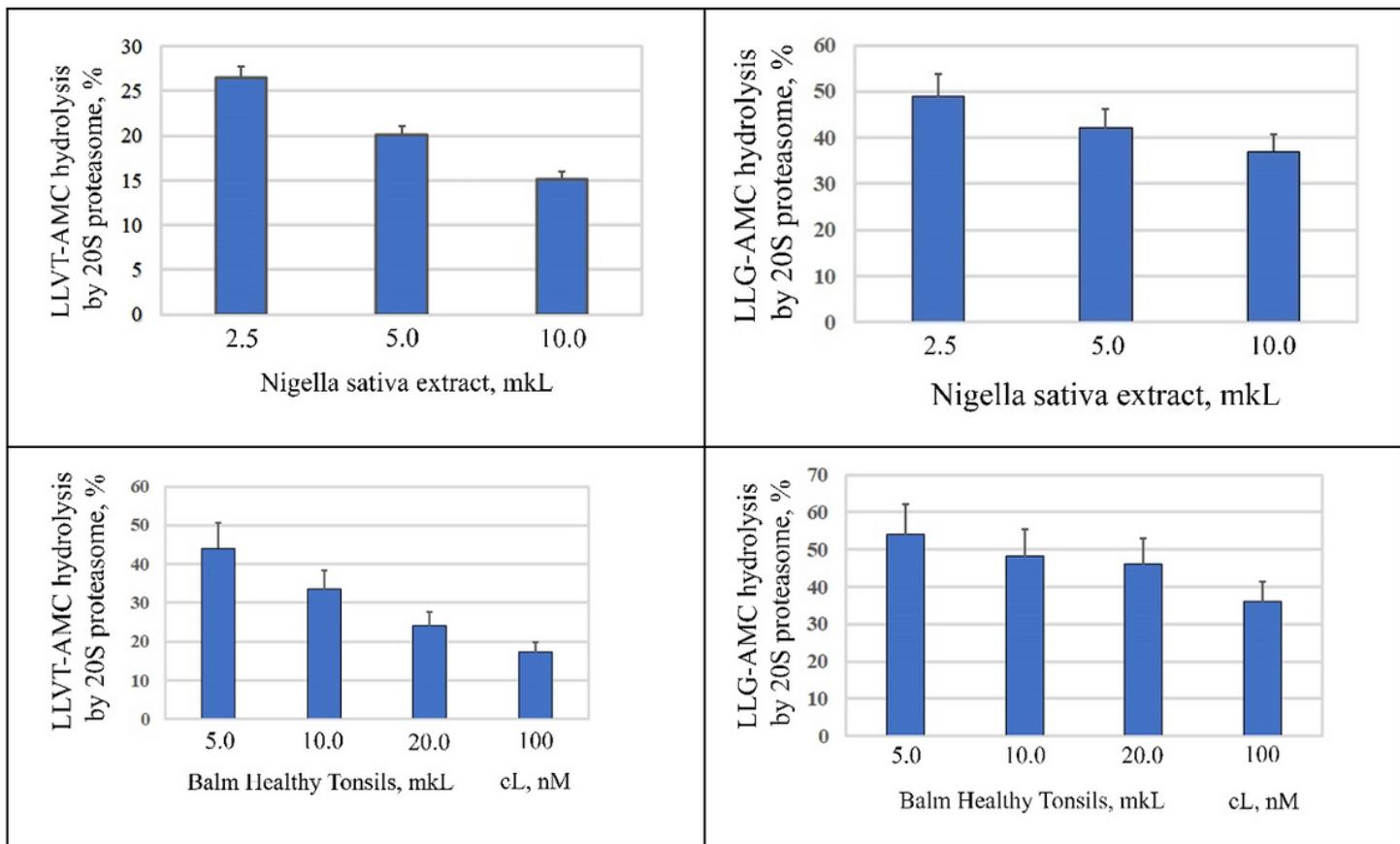


Figure 1

The influence of different concentrations of Nigella sativa extract on chymotrypsin-like (A) and caspase-like (B) activities of purified 20S proteasome. C, D – Changes of chymotrypsin-like and caspase-like activities of the purified proteasomal fraction under the influence of medical phytobalm «Healthy Tonsils».