Comparative description of the anti-inflammatory action of the food concentrate “Enoant” and resveratrol in the model of an acute exudative inflammation

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Abstract. The paper considers comparing the anti-inflammatory action of the food concentrate “Enoant” and a classical antioxidant – resveratrol in the doses of 20 mg/kg in the model of an acute exudative inflammatory of animals. It was proved that resveratrol had the most anti-inflammatory action; this was manifested by a considerable decrease of the increase rate of the animal paw’s size in the formalin test after 7 and 14 days of taking the antioxidant. The food concentrate “Enoant” demonstrated anti-inflammatory properties after 7 days of taking the antioxidant; this was manifested by the increase of the animal paw’s size in the formalin test, however 14 days later a moderate anti-inflammatory reaction was registered, this was manifested by the decrease of the increase rate of the animal paw’s size in the formalin test..

1 Introduction

At present an anti-inflammatory action of many antioxidants is known [1-2]. Antioxidants are able to modify the process of inflammatory reactions and to block many signal molecules, including some genetic mechanisms [3-5].

The effects of different antioxidants have different degree of anti-inflammatory intensity and tropism to different stages of chronic inflammatory process. In connection with this there is a growth of interest in using such multicomponent compositions, in which an antioxidant, and probably anti-inflammatory, action had a cumulative character. One of such polyvalent compositions is a Crimean product – the food concentrate “Enoant”, manufactured by the plant “Magarach”. The sample antioxidant with a proved anti-inflammatory action in different models of inflammation is resveratrol.

The anti-inflammatory action of most antioxidants is demonstrated in the models of chronic lingering inflammation, mainly visceral, which is demanded by the needs of therapeutic practice. However, there is no data in the scientific literature on the anti-inflammatory action of antioxidants in the models of an acute inflammatory.

1.1 Purpose of the study

The purpose of the study was to carry out a comparative analysis of the anti-inflammatory action of the multicomponent food concentrate “Enoant” and resveratrol in the model of an acute exudative inflammatory.

2 Experimental

The research was carried out at the Centre for Collective Use of Scientific Equipment “Experimental Physiology and Biophysics” of V. I. Vernadsky Crimean Federal University.

The research was done on 40 pedigreeless white female rats at the age of 7 months weighed 250 g. Healthy animals were selected for the experiment.

The animals were divided into 2 equal groups of 20 individuals in each. The animals from Group 1 took resveratrol in the isoform of trans-stereoisomer (the producer Solgar) in the dose of 20 mg/kg during two weeks. As resveratrol is poorly dissolved in water, its powder was dissolved in 1 ml of 96 % alcohol and, brought to a homogeneous state, and was added into the drinking tanks of the animals.

The animals from Group 2 took daily the food concentrate “Enoant” in the dose of 20 mg/kg during two weeks. 1 ml of 96 % alcohol was added into the prepared solution. The prepared solution was added into the drinking tanks of the animals. The food concentrate “Enoant” is produced according to technological conditions, standardizing the content of phenolic substances in the product at the level of 18-20 g/dm3, controlled photocalorimetrically by the Folin-Ciocalteu reagent [TC U 00334830.018-99 the food concentrate of grapes polyphenoles “Enoant”, IW&W “Magarach”]. According to the antioxidant action the concentrate “Enoant” excels the antioxidant properties of ascorbic acid by 15 times, and antioxidant properties of blood plasma more than by 3 times [6-8].

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To determine the anti-inflammatory action of the antioxidants all the animals were administered the formalin test before taking the antioxidants, as well as the animals were tested on the 7th day and the 14th day of taking the antioxidants.

Modelling of an acute exudative inflammation was done according to the Handbook on the experimental (pre-clinic) study of pharmacological substances [9]. An acute inflammatory reaction was reproduced by a subplantar introduction of 0.1 ml 2% formalin solution as a water solution into the rat's left hind paw ("the formalin test"), which was prepared before introducing by adding of 17.5 ml of distilled water into 1 ml of 37% formaldehyde solution. The size of the limb's edema was measured by the method of water plethysmometry 50 minutes – an hour later after introducing the formalin, as exactly after the stated time an edema and a pain reaction, caused by the inflammation, develop. This is confirmed by the fact that nonsteroid anti-inflammatory drugs (in particular, ibuprofen, acetylsalicylic acid) suppress the development of this reaction [9].

The degree of the edema after the formalin introduction, proportional to the inflammatory reaction intensity, was evaluated with the help of a digital water plethysmometer LE 7500 meant for measuring small changes of the size. That is why the mentioned equipment can be used for the observation of the development of an experimentally caused inflammation reaction of the rodents and for the evaluation of the anti-inflammatory action of pharmacological drugs.

The animal's paw was put into the chamber of the plethysmometer up to the tibiotarsal joint with the further registration of the paw's size. After that 0.1 ml of 2% formalin solution was introduced into the left hinder paw of the rat, and an hour later after the formalin injection, an iterative measuring of the paw size in the water plethysmograph was done.

The involvement of the researched antioxidant into the inflammatory process regulation was judged by the degree of the increase size of the paw, expressed in the percentage to the initial size. In particular, the increase rate of the paw's size (IR, %) was calculated by the following formula (1):

$$IR=\frac{PSR-IPS}{IPS}*100\% \qquad (1)$$

where IR is the rate of increase of the paw's size an hour later of the formalin injection, expressed by the percentage, PSF is the paw size, registered an hour later after the formalin injection, IPS is the initial paw size, registered before the formalin injection. In this way the increase rate, when taking the food concentrate "Enoant" by the animals – IR(Enoant), as well as when taking resveratrol – IR(res), was evaluated.

The research is carried out according to the State Standard P-53434-2009 «The principles of a proper laboratory practice» and according to the European convention for the protection of the vertebrate animals used for experimental and other scientific purposes.

All the animals were kept under the vivarium conditions at the temperature 18-22oC with a natural 12-hour light-darkness cycle, a free access to water and full-fledged granulated food (State Standard 33215-2014 "The Handbook on keeping and grooming the laboratory animals. The rules for equipping the premises and procedure arrangement"). All the researches were mainly done during the day time 11.00-13.00.

The evaluation of the reliability of inter-group differences of the obtained data was done by Mann-Whitney test. The evaluation of the reliability intergroup differences was done by Friedman test.

The calculations and graphic registration of the obtained during research data were done using the programme Microsoft Excell and the software package "STATISTICA – 8.0".

3 Results and discussion

As the results of this research show, during taking antioxidants by animals the changes in the reactions of the model of an acute exudative inflammation were registered, however these differences had a differently directed character during the first week of the observation, and during the second week the observation was of a unidirectional character, but the differences differed in the degree of intensity.

A. Dynamics of the anti-inflammatory action in the formalin test during taking resveratrol by animals.

Before taking the researched antioxidant by the animals IR at the injection of formalin was 29%. During a daily taking of resveratrol in the dose of 20 mg/kg, a considerable decrease of the inflammatory reaction in the formalin test was observed; this was manifested through the decrease of IR edema of the distal paw section in the dynamics of two-week observation (Table 1).

So, a week later after taking resveratrol the indicator IR of the paw's size after the formalin injection was 10.8%; this is by 62.75% ($p \leq 0,05$) lower of this indicator values, registered before taking the antioxidant.

Fourteen days later after taking resveratrol the level of IR continued decreasing and was 8.49%, this is by 70.72% ($p \leq 0,05$) lower in comparison with the initial data of this indicator, registered before taking the antioxidant.

Thus, the pronounced decrease of the IR indicator of the animal paw's size after injecting formalin in the dynamics of two-week' taking resveratrol evidences of a pronounced anti-inflammatory reaction of the researched substance.

According to the scientific literature data, one of the mechanisms of the nocigenic action of the formalin is the activation of *TRPA1* canals, reacting to the cold in the norm and stimulating the inflammation development, correspondingly, the decrease of the response of the inflammation reaction while doing the formalin test in the dynamics of taking resveratrol evidences of the indirect through blocking *TRPA1*-canals anti-inflammatory mechanism of the action of the researched antioxidant.

Table 1. The increase rate (IR) of the animal paw's size in the formalin test, registered before, and also on the 7th and 14th day of taking resveratrol in the dose of 20 mg/kg

Indicators	Background	7th day	14th day
IR, %	29	10.8 **p≤0.05**	8.49 **p≤0.05**

Note: reliability of differences p≤0.05 by Friedman's test.

B. Dynamics of the anti-inflammatory action in the formalin test during taking the food concentrate "Enoant" by animals.

So, before taking the researched antioxidant by animals, IR of the paw after the formalin injection was 29%. As the research results showed, two-week's taking of the food concentrate "Enoant" was followed by differently directed reactions in the model of an acute exudative inflammation, depending on the duration of taking. Thus, 7 days after taking the antioxidant, the growth of the indicator IR up to the level of 40% was observed, this is 37% $p<0.05$ higher of the values of this indicator, registered before taking the food concentrate (see Table 2). The growth of the indicator of IR of the animal paw's size in the formalin test demonstrates the increase of the reaction of an acute exudative inflammation in the formalin test.

But two-week's taking of the food concentrate "Enoant" in the dose of 20 mg/kg was followed by the change of the operational sign – the lowering of the indicator IR of the animal paw's size in the formalin test up to the level of 22 %, which is by 24.14 % $p<0.05$ lower than the values of this indicator, registered before taking the food concentrate; this evidences of the anti-inflammatory action of the researched antioxidant during a more prolonged taking in the model of an acute exudative inflammation.

Based on the obtained results, the antioxidant complex shows differently directed action in the model of an acute exudative inflammation. In the first place, a pro-inflammatory orientation is observed, and in the second place – an anti-inflammatory one. Probably, the pro-inflammatory action, registered in the initial period of the food concentrate, is conditioned by the activation of the components of the acute phase of the inflammation, aimed at the liquidation of phlogogen, including the activation of chemotaxis factors, phagocytes migration to the nidus and further lysis of phlogogene with the subsequent regeneration. Evidently, such a reaction in the model of an acute exudative inflammation is physiological and provides an effective elimination of the factor, having provoked the inflammation. The effectiveness of the proinflammatory phase is evidenced by the occurrence of the second anti-inflammatory phase, being registered during a prolonged taking of the antioxidant complex. We may assume that a successful solution to the acute inflammation prevents the inflammatory process from becoming chronic.

Table II. The increase rate (IR) of the animal paw's size in the formalin test, registered before, and also on the 7th and 14th day of taking the food concentrate "Enoant" in the dose of 20 mg/kg

Indicators	Background	7th day	14th day
IR, %	29	40 **p<0.05**	22 **p<0.05**

Note: reliability of differences $p<0.05$ by Friedman's test.

C. A comparative evaluation of the anti-inflammatory action of resveratrol and the food concentrate "Enoant" in the model of an acute exudative stress.

The results of the given research evidence of different mechanisms of the anti-inflammatory action of the antioxidants under research. Thus, after taking resveratrol by animals, the pronounced decrease of the inflammatory reaction was noted; this reaction was registered a week later after taking the antioxidant, and it was less expressed two weeks later after taking this substance. At the time when in the animals, taking the food concentrate "Enoant" in the model of an acute exudative stress, the growth of pro-inflammatory reaction was registered a week later, as well as a moderate lowering of the anti-inflammatory reaction two weeks later after taking the concentrate.

According to the scientific literature data, the mechanism of the anti-inflammatory effect of resveratrol is implemented through the activation of different protein molecules of sirtuins. Stimulating sirtuins *Sirt3* and *Sirt1*, resveratrol has an indirect influence on the proteins of *FOXO* (*forkhead box O*) family. These proteins are transcriptional factors, controlling the expression of genes, responsible for proliferation, differentiation, apoptosis and reaction to the external stresses. Regulating the expression of target genes, *FOXO* protect the organism from unfavourable external influences, activate protective mechanisms and, ipso facto, slow down the aging processes [10]. So, taking resveratrol is accompanied by the anti-inflammatory action due to the lowering of the expression of the nuclear transcriptional factor *cB (NF-cB*), participating in the transcription of the anti-inflammatory genes, encrypting cytokines and adhesion molecules [5,11].

The chemical composition of the food concentrate "Enoant" is versatile. Thus, examining the composition of the polyphenol product, we may mention the presence of a number of monomeric flavonoids, such as cvercetin, rutin and their derivatives, such as catechine, epicatechin, epichatechin gallate. Both rutin and cvercetin have not only a pronounced antioxidant action, but also an anti-inflammatory action, this allows using them in the therapy of an amount of chronic inflammatory diseases. The anti-inflammatory rutin properties are manifested in the suppression of the transcription of pro-inflammatory cytokines [12]. Besides, rutin inhibits metabolism of arachidic acid – the predecessors of inflammation mediators [13], levels the synthesis of tumor's necrosis factor [14].

The anti-inflammatory properties of cvercitine are demonstrated through the suppression of inflammation mediators synthesis, anti-inflammatory cytokines [15], through the blockade of synthesis leukotrienes, inhibition of phospholipase A_2 [16], activity lowering of inducible NO-synthase, inhibition of the acute inflammation phase, as a consequence of apoptosis suppression [17].

Besides, the composition of the food concentrate "Enoant" includes monomeric flavonoids (their concentration exceeded the same, registered in the wine of "Cabernet" type, approximately by 6 times). The main part of monomeric anthocyanins is presented by malvidin glycoside and its derivatives. Thereby, the food concentrate "Enoant" is a complex food concentrate, in which many of its components can work in synergism.

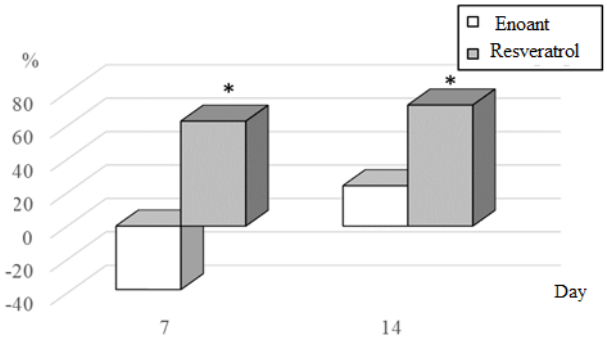

Fig.1 The dynamics of the anti-inflammatory action rate expressed in % in comparison with the initial data of this indicator in the model of the acute exudative inflammation, registered in the animals while taking resveratrol and the food concentrate "Enoant" in the dynamics of two-week's observation.

Note: reliability of differences* - $p<0.05$ by Mann-Whitney test

Thus, it is possible to conclude that resveratrol inhibits sharply the activity of anti-inflammatroy reactions and signal inflammatory molecules, while the food concentrate "Enoant" meditates its anti-inflammatory action through the activation of pro-inflammation, this is reasonable for phlogogene liquidation, after that an anti-inflammatory action is demonstrated. We may suggest that the anti-inflammatory mechanism of the food concentrate "Enoant" action is more physiological and soft.

4 Conclusion

1. The anti-inflammatory effects of resveratrol and the food concentrate "Enoant" in the equivalent doses of 20 mg/kg in the model of acute exudative inflammation are shown.
2. Taking resveratrol in the dose of 20 mg/kg contributed to the reduction of the inflammatory reaction, this was manifested through the decrease of the increase rate of paw's size in the formalin test model by 62.75% ($p\leq0.05$) after 7 days of taking the antioxidant and by 70.72% ($p\leq0.05$) after 14 days of taking the antioxidant in comparison with the values of this indicator, registered before the taking. Taking the food concentrate "Enoant" in the dose of 20 mg/kg contributed to the intensification of the inflammatory reaction, this was manifested through the growth of the increase rate of paw's size in the formalin test model by 37 % ($p\leq0.05$); after 14 days of taking the antioxidant the reduction of the inflammatory reaction was noticed, this was manifested through the decrease of the increase rate of paw's size in the formalin test model by 24.14% ($p\leq0.05$) in comparison with the values of this indicators, registered before taking the antioxidant.

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