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STUDY OF THE ANTI-INFLAMMATORY EFFECT OF TERPENOIDS

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Carvone and verbenone are natural organic substances that belong to the monoterpene class terpenoids, which are quite common in nature and are contained in many herbal medicines. Today, it is quite relevant to study the effectiveness of the anti-inflammatory effect of carvone and verbenone, as well as to compare their anti-inflammatory action. The effect of carvone and verbenone on seroglycoids content in rat blood serum and their anti-inflammatory action in the model of carrageenan-induced inflammation was studied in the work presented. The development of edema under the carvone and verbenone action against the background of an inflammatory reaction was evaluated and the results of the increase in the volume of the lesion and the anti-edematous action of the studied substances were compared, which reflects the process of suppression of the pathological reaction in animals of the experimental groups compared to the control one. The study of blood serum seroglycoside content in carrageenan-induced inflammation revealed a significant suppressive effect of both test substances on increasing the concentration of seroglycoids (only by 27.5 % and 42.5 %, respectively) at the initial stage of the pathological process, which may indicate a less active development of the inflammatory process during this period. The anti-inflammatory action of carvone throughout the study was slightly better than that of verbenone. Besides, the results of the study on the model of carrageenan-induced inflammation indicate the effective anti-edematous action of both carvone and verbenone. The effect of verbenone had a fairly stable anti-inflammatory action throughout the entire period of the experimental study (from 7.4 % up to 62.4 %). However, the most significant was the anti-inflammatory action of carvone, especially in the second half of the study (from 28.6% up to 100%). Significant anti-inflammatory action of carvone was established since the first day of the study, and at the end of the study, the morphological parameters of the affected rats' limbs returned to their original state. The obtained study results allow us to state the effective anti-inflammatory action of both carvone and verbenone.

Keywords: *carrageenan-induced inflammation, carvone, verbenone, anti-inflammatory action.*

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ДОСЛІДЖЕННЯ ПРОТИЗАПАЛЬНОЇ ДІЇ ТЕРПЕНОЇДІВ

Карвон і вербенон природні органічні речовин, які відносяться до терпеноїдів монотерпенового ряду, що досить поширені у природі і містяться в багатьох лікарських препаратах рослинного походження. На сьогодні є досить актуальним дослідження ефективності протизапальної дії карвону та вербенону, а також порівняння їх протизапальної активності. У роботі досліджено вплив карвону та вербенону на вміст сироглікоїдів у сироватці крові щурів та їх протизапальну дію на моделі каррагінан-індукованого запалення карвону. Проведено оцінку розвитку набряку за дії карвону і

вербенону на фоні запальної реакції та порівняльний аналіз результатів приросту об'єму ураження і протинабрякової активності досліджуваних речовин, що є відображанням процесу пригнічення патологічної реакції у тварин дослідних груп, в порівнянні з контрольною групою. В ході дослідження вмісту сіроглікоїдів сироватки крові за каррагінан-індукованого запалення було встановлено значну пригнічувальну дію обидвох досліджуваних речовин щодо збільшення концентрації сіроглікоїдів (лише на 27,5 % та 42,5 %, відповідно) на початковій стадії розвитку патологічного процесу, що може свідчити про менш активний розвиток запального процесу в цей період. Протизапальний ефект карвону в ході всього дослідження були трохи кращим, порівняно з впливом вербенону. Також, отримані результати дослідження на моделі каррагінан-індукованого запалення свідчать про ефективну протинабрякову активність і карвону, і вербенону. Вплив вербенону мав досить стабільний протизапальний ефект протягом всього періоду експериментального дослідження (від 7,4 % до 62,4 %). Та найбільш суттєвою була протизапальна дія карвону, особливо в другій половині дослідження (від 28,6 % до 100 %). Значна протизапальна активність карвону була встановлена з першого дня дослідження, а під кінець – спостерігалося повернення морфологічних показників уражених кінцівок щурів до вихідного стану. Отримані результати дослідження дозволяють говорити про ефективну протизапальну активність як карвону, так і вербенону.

Ключові слова: *каррагінан-індуковане запалення, карвон, вербенон, протизапальна дія.*

Carvone and verbenone are natural organic substances that belong to the monoterpene class terpenoids, which are quite common in nature. They are especially abundant in plants. Biologically active substances are less toxic than their synthetic counterparts, so they cause significantly fewer adverse reactions. Currently, there are many herbal medicines based on terpenes and terpenoids that can simultaneously exhibit significant biological activity and, to some extent, not harm organisms.

When analyzing the literature, the most commonly encountered studies are those on the pharmacological effects and properties of various essential oils containing mixtures of terpenes and terpenoids, including carvone and verbenone. Essential oils containing verbenone have anti-inflammatory and antispasmodic action, possessing analgesic and antiseptic properties [1, 2, 3]. Studies on the effect of verbenone and its derivatives on various biological processes and living organisms are also found in the literature [4, 5, 6], however, in a much fewer number.

Carvone or essential oils with its high content have a fairly wide range of applications in various industries, especially in medicine. Herbal medicines with a high carvone content are often used to treat or prevent diseases [7, 8]. Various pharmaceuticals contain isomeric forms of carvone or its derivatives [9, 10]. Significant antioxidant and anti-inflammatory properties of carvone have been established [11, 12]. Carvone is used in veterinary medicine as an effective anti-inflammatory agent for ulcerative colitis [13] and gastrointestinal flatulence [14, 15]. The literature contains many studies of the anti-inflammatory action of carvone, however, along with its anti-inflammatory action, it can cause the development of negative side effects [10, 16, 17].

Thus, today it is quite relevant to study the effectiveness of the anti-inflammatory action of carvone and verbenone, as well as to compare their anti-inflammatory action.

The objective of the study was to determine the effect of carvone and verbenone on seroglycoids content in rats' blood serum and their anti-edematous effect on carrageenan-induced inflammation, to compare their anti-inflammatory action.

MATERIALS AND METHODOLOGY

The studies were conducted on male mature white rats weighing 200-250 g. The experimental studies were conducted in accordance with requirements of the European Convention for the Protection of Animals [18] and the guidelines for animal care [19]. Animals were divided into three groups, control and two experimental groups, respectively, 6 animals each. A model of carrageenan-induced inflammation was created in rats, against which the development of an inflammatory response was monitored in animals of the control group during the study (control). Animals of the first experimental group were orally administered 1 ml of verbenone solution once a day, and animals of the second experimental group were administered 1 ml of carvone solution at a dosage of 100 mg/kg.

Carrageenan-induced inflammatory process in rats was modeled by a single subplantar injection of 0.1 ml of 0.2 % carrageenan solution under the plantar aponeurosis of the back left paw of the animal. The first injection of the studied carvone and verbenone solutions was performed before the development of an inflammatory reaction after the phlogogenic agent administration. To evaluate the development of edema, the method of measuring the volume of animals' paws using a plethysmometer before the phlogogenic agent administration (baseline data), at the time of the maximum development of the inflammatory response (6 hours after the carrageenan administration) and every 24 hours during 6 days of the study, was used [20, 21]. The results of the experimental study of the volume of the affected limbs were expressed as a percentage relative to the baseline indicators (intact animals). The relevant formulas were used to calculate the volume gain and decongestant effect [22]. The seroglycoids content in the blood serum of rats was determined using a standard set of reagents "FDelicid" (for determination of blood serum seroglycosides by the turbidimetric method). The seroglycoids content was determined in units of turbidity (S-H) using the data of the calibration graph [23].

Statistical processing of the study results was performed using the Student's coefficient [24].

RESULTS AND DISCUSSION

The carrageenan-induced inflammation model is one of the methods for reproducing the experimental inflammatory process. Seromucoids are complex acidic glycoproteins, which include a carbohydrate component, and are acute-phase proteins, long-known markers for prognosis and course of various diseases. Seroglycoids content in blood serum can rise sharply even before other signs of the

pathological process appear and indicate the development of inflammation of various etiologies. It is known from the literature that seroglycoids can be quite sensitive markers of both acute and chronic inflammatory processes [25, 26, 27, 28], markers of connective tissue destruction, as they are its normal constituent elements, etc.

Within the course of the study, a sharp increase in the seroglycoids content by 127.5% was observed in the control group from the first day, compared to the baseline. Subsequently, the increase in seroglycoid content in this group gradually decreased, but remained significantly high until the end of the study. Thus, in the middle and at the end of the study, the blood serum seroglycosides content was 60.0% and 50.0%, respectively, compared with the baseline values (Fig. 1).

An increase in the amount of seroglycoids was also found in the blood serum of the experimental groups of animals treated with verbenone (group 1) and carvone (group 2) in carrageenan-induced inflammation, but it had a different developmental pattern. In the experimental groups, there was no sharp increase in the content of seroglycoids on the first day of the study, similar to the results of the control group. Under the condition of carvone and verbenone action on the model of carrageenan-induced inflammation, the amount of blood serum seroglycoids on the first day of the study increased by only 27.5% and 42.5%, respectively, compared to the baseline. However, on the third day of the study, the seroglycoids content in the blood serum of the experimental group of animals increased compared to the baseline values. Thus, according to the study results, in the experimental group of animals treated with carvone for carrageenan-induced inflammation, an increase in the content of seroglycoides by 105.0% was found, and in the group of animals treated with verbenone, the increase was 115.0% compared to the baseline, which may indicate a gradual development and exacerbation of the inflammatory process. On the sixth day of the study, there was a decrease in the amount of seroglycoids in the blood serum of the experimental groups under the influence of carvone and verbenone on the model of carrageenan-induced inflammation and, compared to the baseline, was 37.5% and 45.0% higher, respectively (Fig. 1).

Thus, the study revealed a gradual increase and decrease in seroglycoids content in the blood serum under both carvone and verbenone effects in the carrageenan-induced inflammation model. At the beginning of the study, there was an inhibition of seroglycoids production under the influence of carvone and verbenone, which may indicate a less active development of the inflammatory process within this period. If we compare the carvone and verbenone action, it should be noted that the anti-inflammatory action of carvone in the study was slightly better compared to the results obtained under the action of verbenone.

At the injection area, after carrageenan administration, the pathological process is actively developing, swelling and pain appear rather quickly, due to the active release of cellular and humoral inflammatory mediators. An increase in the volume of the affected limbs of rats was recorded within the first hours after exposure to the phlogogenic factor. On the first day of the experimental study, the volume of the affected limbs in the control group of animals increased by 68.8 % compared to the

baseline values. On the second day, the progression of the pathological process and a further increase in the volume of the affected limbs by 87.5% were observed. Starting from the third to the fifth day, the swelling of the affected limbs reduced, but remained consistently high at 50.0% compared to the baseline values. Further suppression of the inflammatory process was observed on the sixth day of the study. The affected limbs volume was 25.0% higher copmpared to the baseline values.

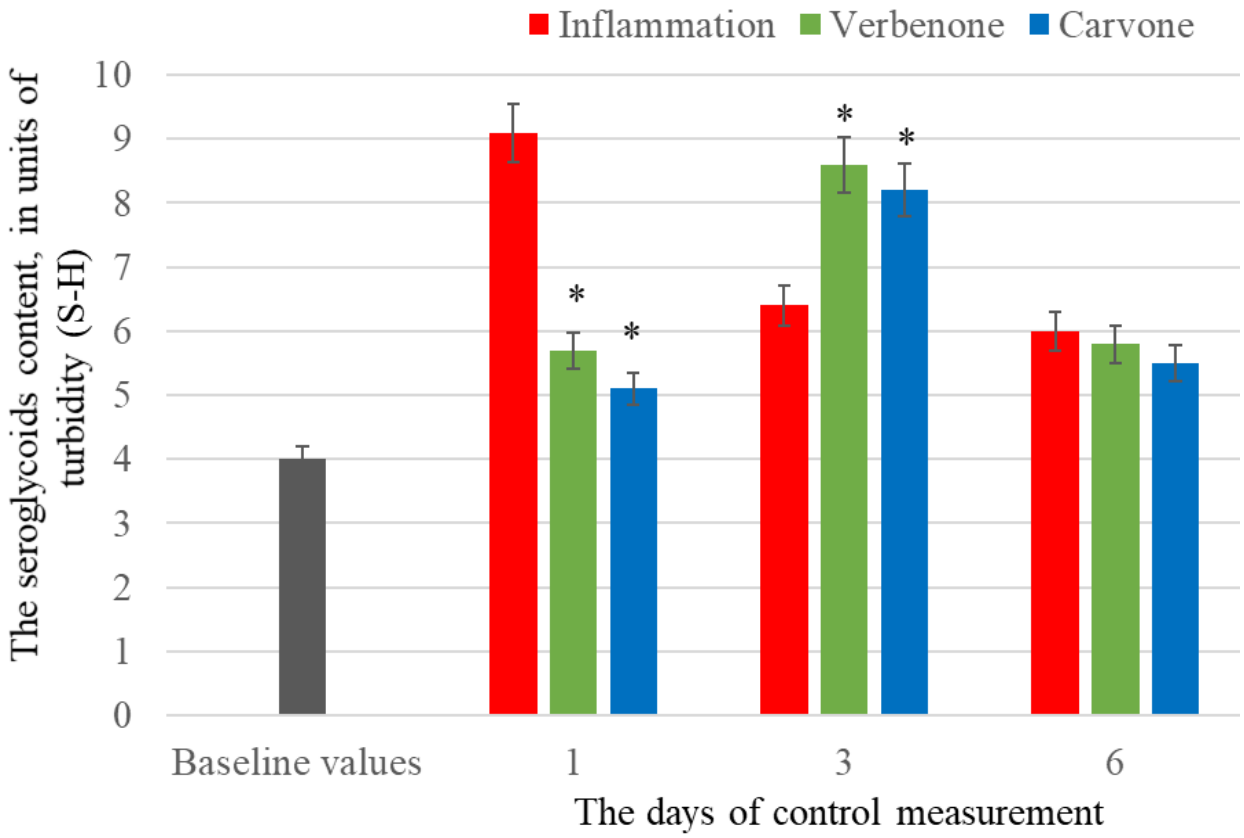

Fig. 1. The siroglycoids content in blood serum under the influence of carvone and verbenone on the model of carrageenan-induced inflammation

Notes: * – degree of reliability of indicators ($p<0.05$) relative to the control
baseline indicators – data obtained before the phlogogenic agent administration and taken as 100%
1 – 6 hours after the phlogogenic agent administration
3, 6 – every 48 hours during the study

An increase in the volume of the affected limbs was also observed with the experimental groups of animals that were orally administered 1 ml of 0.2% verbenone (group 1) and carvone (group 2) solution daily against the background of carrageenan-induced inflammation. However, the development of an inflammatory reaction and edema were less pronounced. Thus, within the first two days of the study, i.e., the acute phase, the increase in the affected limbs volume of the rats in the first experimental group was 42.5% and 66.3%, respectively, compared to the baseline. The swelling of the affected limbs in the second experimental group

increased by only 37.5% and 62.5%, respectively, compared to the baseline values (Fig. 2).

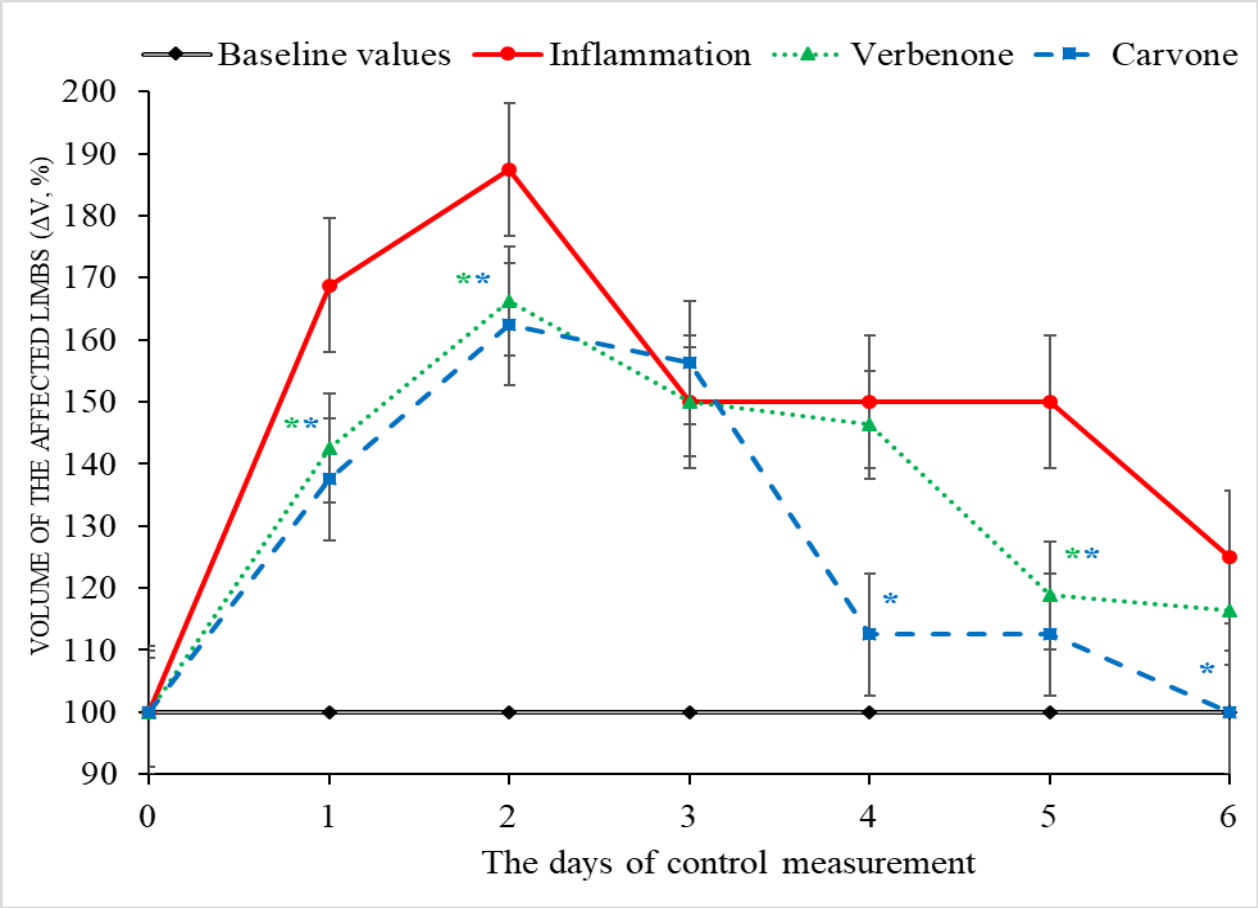

Fig. 2. Volume of the affected limbs of animals under the influence of carvone and verbenone on the model of carrageenan-induced inflammation, in % compared to baseline

Notes: * – degree of reliability of indicators ($p<0.05$) relative to the control
0 – before the phlogogenic agent administration (initial data taken as 100%)
1 – 6 hours after the phlogogenic agent administration
2-6 – every 24 hours during the study

A decrease in the inflammatory process was noted on the third day of the study. The increase in the affected limbs volume in the experimental groups was actually the same as in the control group and amounted to 50.0% under the action of verbenone and 56.3% under the action of carvone, compared to the baseline values.

A gradual attenuation of the inflammatory process and a decrease in edema in animals of the first experimental group treated with verbenone was found in the second half of the experimental study. The increase in the affected limbs volume on the fourth, fifth and sixth days was 46.3%, 18.8% and 16.3%, respectively, compared to the baseline. That is, a more pronounced anti-inflammatory effect of verbenone was observed on the fifth and sixth days. As to animals of the second experimental group, which were injected with carvone, the suppression of the inflammatory response was more pronounced on the fourth and fifth days. The swelling of the

affected limbs was only 12.5% greater compared to the baseline values. Moreover, on the sixth day of the study, the affected limbs volume of the experimental animals was at the level of the baseline values.

The analysis of the study results obtained under the influence of verbenone suggests that its anti-edematous action was quite good, which was observed throughout the study period. Thus, on the first day, the anti-edematous action of verbenone, i.e., the increase in the affected limbs volume with animals of the first experimental group, compared to the control group, was 38.2%. On the second day of the study, when the control group showed the greatest swelling of the affected limbs, the anti-edematous action of verbenone was 24.2%. On the third day of the study, the swelling of the affected limbs in both groups was the same, and the anti-edema action was zero. The anti-edematous action of verbenone on the fourth day of the study was also insignificant and amounted to only 7.4%. But on the fifth and sixth day of the study, the anti-edematous action of verbenone increased significantly and amounted to 62.4% and 34.8%, respectively.

The most significant anti-inflammatory action of carvone was found on the first day and the second half of the study. Thus, on the first day of the study, the increase in the affected limbs volume of animals of the second experimental group was 45.5% lower than in the control one. On the second day, when the control group recorded the highest increase in the affected limbs volume, the anti-edematous action of carvone was 28.6%. Starting from the third day and until the end of the study, the anti-edematous effect of carvone increased significantly and ranged from 75.0 up to 100%. That is, on the last day of the study, the morphological indicators of the affected limbs returned to their original state, respectively, the anti-edematous action and efficiency of carvone application was 100%.

The literature sources report a significant anti-inflammatory action of both verbenone and its derivatives [1, 2, 3, 5, 6], as well as carvone [12, 29]. The anti-inflammatory action of plant extracts containing verbenone is manifested in a significant inhibition of lipid peroxidation, suppressed production of nitric oxide and tumor necrosis factor TNF-α, suppressed expression of nitric oxide synthases and cyclohexokinase-2, the universal transcription factor NF-κB, which controls the expression of genes of the body's immune response, apoptosis, and cell cycle [2, 3, 5, 6], and stimulation of a significant increase in the level of interleukin-6, which can act as an anti-inflammatory cytokine [4]. As for carvone, its effect is associated with a decrease in the alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activity in the blood serum, an increase in glutathione levels, a decrease in the expression of TNF-α, IL-1 and IL-6, and infiltration of inflammatory cells [11]. Carvone has an anti-inflammatory action, manifested in the suppression of bradykinin, histamine, prostaglandin E2, serotonin, interleukin-1 and TNF-α, and increased glutathione levels [12], as well as a decrease in anti-inflammatory cytokines, interleukins and other markers of oxidative stress [30] is observed.

The obtained study results on the model of carrageenan-induced inflammation indicate the effective anti-edematous action of both test substances. The effect of

verbenone had a fairly stable anti-inflammatory action throughout the entire period of the experimental study. But the most significant was the anti-inflammatory action of carvone, especially in the second half of the study. The results of study of the seroglycoids content in the blood serum under carrageenan-induced inflammation indicate a significant suppressive effect of carvone and verbenone on increasing their concentration at the initial stage of the pathological process. The anti-inflammatory action of carvone throughout the study was slightly better than that of verbenone.

CONCLUSIONS

The obtained study results suggest an effective anti-inflammatory action of both carvone and verbenone. The study of seroglycoids content in the blood serum under carrageenan-induced inflammation revealed a significant suppressive effect of both carvone and verbenone on increasing their concentration at the initial stage of the pathological process. In the model of carrageenan-induced inflammation on the first day of the study, a sharp increase in the seroglycoids content by 127.5% was observed, and under the action of carvone and verbenone - only by 27.5% and 42.5%, respectively, compared to the baseline values. Subsequently, a gradual increase and decrease of seroglycoids content in the blood serum was found under both carvone and verbenone effects, however, the results of the anti-inflammatory action of carvone in the study were slightly better compared to that of verbenone.

When studying the anti-inflammatory action of verbenone on the model of carrageenan-induced inflammation, its rather stable anti-edematous action (from 7.4 % up to 62.4 %) was found, which was observed throughout the entire period of the experimental study and was most pronounced in the acute phase and the period of the inflammatory process attenuation. However, the most effective anti-edematous activity was found under the condition of carvone action during carrageenan-induced inflammation, which ranged from 28.6 % up to 100 %. A significant anti-inflammatory action of carvone was established since the first day of the study, and at the end of the study, the morphological indicators of the affected limbs returned to their original state.

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