

The Study of Antiallergic Activity of the Phytocollection of “Phytoallergoderm”

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Abstract

The antiallergic activity of “Phytoallergoderm” phytocollection was studied, which was recommended for topical treatment of a topic dermatitis, on the model of allergic contact dermatitis, caused by skin application of turpentine. The following drugs were used as comparison: antihistamine “Psilo-Balsam” gel, manufacturer: OJSC “Nizhpharm” Russia; glucocorticoid (topical steroid) “Dermoveit” cream 0.05%, manufacturer: “Glaxo Smith Kline Pharmaceuticals SA” Poland; zinc product “Zinc” ointment, manufacturer: NPP “Radiks” Uzbekistan.

During the experiment, it was found that the test drug has a reliable anti-allergic activity, which is clearly caused of decrease in total allergic reactions, in the reduction of recovery time and in a decrease in the dynamics of the severity of allergic reactions. Also, the study of antiallergic activity showed that the test drug has a comparable antiallergic activity with the etalon synthetic drug.

The foregoing indicates the presence of a reliable anti-allergic activity of the phytocollection “Phytoallergoderm”, which is comparable to reference synthetic preparations.

Keywords: *Atopic dermatitis, experimental allergic contact dermatitis, turpentine, phytocollection.*

Introduction

Atopic dermatitis (ATD) is a chronic allergic skin disease with a genetic predisposition and complex development mechanisms that lead to impaired immune response, the development of allergic inflammation and the appearance of a specific symptom complex, which is characterized by age-related features of the localization and morphology of the inflammation hearth, as well as excruciating skin itching¹. ATD is a socially significant problem due to its wide prevalence, as well as a significant impact on the quality of life, limitation of working capacity up to disability (Elisyutina, 2014).²

Today, glucocorticosteroids are widely used in the external treatment of atopic dermatitis, which, despite their effectiveness, have a number of side effects and often cause complications in the form of pyoderma (Elisyutina, 2014 and Skripkin, 2000).³ However, the treatment of skin diseases with medicinal plants remains a promising direction, since medicinal plants act in a complex manner, and being non-toxic rarely

cause adverse reactions (Skripkin, 2000 and Imamaliyev, 2015)⁴.

Based on the foregoing, we have developed a phytocomplex (phytocollection) “Phytoallergoderm”, recommended for external treatment of a topic dermatitis, and having the following composition: tricolor violet grass; hop cones; licorice roots and; flowers of chamomile pharmacy, the leaves of nettles; flowers of marigolds medicinal; the grass of horsetail (Kochueva, 2014)⁵.

The aim of the study was to investigate the antiallergic activity of phytocollection “Phytoallergoderm”, recommended for the local treatment of atopic dermatitis⁶.

Materials and Method

The study was carried out in accordance with the requirements of the current GLP regional standard-“good laboratory practice”, as well as in compliance with all the rules and regulations of the “European

Convention for the Protection of Vertebrate Animals used for experimental and scientific purposes". In the study, were used healthy animals, that have passed their quarantine for at least 10-14 days.

A study of the anti-allergic activity of the drug was carried out on a model of allergic contact dermatitis caused by skin application of turpentine (turpentine oil) (Kochueva, 2014). Turpentine is known to be an industrial allergen, and external exposure to turpentine causes a strong allergic reaction, including dermatitis. It is known that when an allergen is exposed, histamine and a number of other inflammatory mediators are released, which contribute to the formation of an allergic reaction.

As comparison drugs (control drugs) were used:

- Antihistamine "Psilo-Balsam" gel for external use 1% in 20 g (tube), manufacturer: OJSC Nizhpharm, Russia, active ingredient: Diphenhydramine 1%;
- Glucocorticoid product (topical steroid) "Dermoveit" cream 0.05%, 25 g each (tubes), manufacturer: "Glaxo Smith Kline Pharmaceuticals SA" Poland, active ingredient: Clobetasol 0.05%;
- Zinc product "Zinc" ointment, manufacturer: "Radiks" Company, Uzbekistan, active ingredient: Zinc oxide 10%.

To study the antiallergic activity, the tincture was prepared the collection by GF-XI, in the ratios of 1:5, 1:10, 1:15 (the water was added taking into account the water absorption coefficient $CV = 2.5 \text{ ml/g}$). The solids content of the infusion made up 2.97% (1: 5), 1.96% (1:10) and 1.47% (1:15) respectively.

The experiments were carried out on 60 white mice (both sexes) with a body weight of 18-22 g, which were divided into groups of 6 animals in each. In the experiment were used only healthy animals that have passed quarantine for at least 10-14 days.

To do this, turpentine was applied to the animals daily and once for six days on pre-depilated skin of the right femoral part (hair was cut out a day before the experiment), the depilation area was 2.25 cm^2 ($1.5 \times 1.5 \text{ cm}$). Turpentine was applied in the form of a moistened cotton swab, while the exposure diameter was in the form of a circle, with a diameter of about 1 cm and an area about 0.785 cm^2 . As a result, pronounced morphological changes in the skin were observed in the animals, accompanied by skin itching, anxiety, lichenization, and hyperkeratosis.

Then, on the next day after the last application of turpentine, the animals of the test groups and comparison groups were injected once and daily with drugs until complete recovery:

1. Control group (control) - animals without treatment;
2. Test group No. 1 – the affected skin of the animals was smeared with 2.97% infusion of phytocollection (through a cotton swab), at a dose of 445.5 mg/kg (0.3 ml/20 g);
3. Test group No. 2 – the affected skin of the animals was smeared with 1.96% infusion of phytocollection (through a cotton swab), at a dose of 94 mg/kg (0.3 ml/20 g);
4. Test group No. 3 - the affected skin of the animals was smeared with 1.47% infusion of phytocollection (through a cotton swab), at a dose of 220.5 mg/kg (0.3 ml/20 g);
5. Comparison group (reference group) No. 1 - 1% Psilo-Balm gel was applied to the affected skin at a dose of 35 mg/kg (0.07 g / 20 g);
6. Comparison group (reference group) No. 2 - on the affected skin was smeared with 0.5% Psilo-Balm gel, at a dose of 18 mg/kg (0.07 g/20 g);
7. Comparison group (reference group) No. 3 - 0.05% Dermoveit cream was applied to the affected skin at a dose of 1.75 mg/kg (0.07 g/20 g);
8. Comparison group (reference group) No. 4 - 0.025% Dermoveit cream was applied to the affected skin at a dose of 0,88 mg/kg (0.07 g/20 g);
9. Comparison group (reference group) No. 5 - 10% Zinc ointment was applied to the affected skin at a dose of 350 mg/kg (0.07 g/20 g);
10. Comparison group (reference group) No. 6 - 5% Zinc ointment was applied to the affected skin at a dose of 175 mg/kg (0.07 g/20 g);

During the experiment, a skin condition was assessed daily, according to the severity of inflammation and skin reaction. The severity of signs of inflammation (hyperemia, edema, pain) was evaluated in arbitrary units:

0 – absence of visible reactions;

1 – pale pink erythema throughout the site or along its periphery;

2 – bright pink erythema throughout the site or along its periphery;

3 – red erythema throughout the site;

4 – skin infiltration and edema (thickening of the skin fold) in the presence or absence of erythema;

5 – erythema, severe infiltration, about local ulceration (necrosis), hemorrhages, about the formation of crusts are possible.

The criterion for assessing pharmacological activity was:

- Reduction in the amount of an allergic reaction (inflammation), compared with the control;
- Reduction in the recovery time of animals, compared with control;
- A decrease in the dynamics of an allergic reaction (inflammation), as compared with control.

To assess the amount of an allergic reaction, after the experiment was completed, the skin reaction scores for each animal were summarized, observed throughout the entire period of the experiment (during the treatment period), and the antiallergic activity of the drug was judged by the amount received.

When conducting the experiment, the room temperature is in the range of 18-25⁰ C and the relative humidity in the range of 40-70%.

The results were processed by the method of variation statistics according to the Student criterion at p= 0.05 (Khabrieva, 2005 and Mirobov, 2012). The tables show the arithmetic mean values (M), the corresponding standard error of the mean value (m), student criterion (t), the number of samples (n), and confidence limits (lower confidence limit/upper confidence limit).

Results

When assessing the changes in total allergic reaction, it was found (Table 1) that the test drug in doses of 445.5 mg/kg (54.1%), 294 mg/kg (62.8%) and 220.5 mg/kg (56, 4%) had a reliable antiallergic effect. In this case, the highest antiallergic effect in the test drug was observed at a dose of 294 mg/kg.

In the case of preparation “Psilo Balm” at doses of 35 mg/kg (47, 6 %) and 18 mg/kg (51.2%) also observed a significant anti-allergic effect. At the same time, the highest anti-allergic effect of the drug “Psilo-Balm” was observed at a dose of 18 mg/kg.

In the case of the drug “Dermoveit” at a dose of 1.75 mg/kg (50.0%), a significant anti-allergic effect is observed. However, at a dose of 0.88 mg/kg (12.6 %), although an antiallergic effect was observed, it was not significant. Moreover, the highest antiallergic effect of the drug “Dermoveit” was observed at a dose of 1.75 mg/kg.

Table 1: Results study of antiallergic activity (according to the total allergic reaction) (M±tm; p = 0.05; n = 6)

Group	Result	% effect
The control	41.00 (34.07÷ 47.93)	-
“Fitosbor” (at a dose of 445.5 mg/kg)	19.17 (14.84÷ 23.49)	54.1 %
“Fitosbor” (at a dose of 294 mg/kg)	18.67 (15.24÷ 22.09)	62.8%
“Fitosbor” (at a dose of 220.5 mg/kg)	21.33 (13.21÷ 29.45)	56.4 %
“Psilo-Balm” (at a dose of 35 mg/kg)	21.50 (16.63÷ 26.37)	47, 6 %
Psilo-Balm (at a dose of 18 mg/kg)	20.00 (14.29÷ 25.71)	51.2 %
Dermoveit (at a dose of 1.75 mg/kg)	20.50 (14.88÷ 26.12)	50, 0 %
Dermoveit (at a dose of 0, 88 mg/kg)	35, 83 (27.69÷ 43, 97)	12.6 %
“Zinc ointment “ (at a dose of 350 mg/kg)	17.17 (14.02÷ 20.31)	58.1%
“Zinc ointment “ (at a dose of 175 mg/kg)	17.17 (13.11÷ 21.23)	58.1%

In the case of “Zinc Ointment” at doses of 350 mg/kg (58.1%) and 175 mg/kg (58.1%) also observed a significant anti-allergic effect. Meanwhile, the highest anti-allergic effect of the drug “Zinc Ointment” was observed in both doses of 350 mg/kg and 175 mg/kg.

When the experimental data on the decrease in the total allergic reaction of the most effective doses of the test drug compared with the most effective doses of the comparison drugs, it turned out that the difference between them was not statistically significant.

When assessing the change in the timing of recovery, it was found (Table 2) that the test drug in doses of 445.5 mg/kg (40.6%), 294 mg/kg (48.7%) and 220.5 mg/kg (45.9 %) significantly reduces the recovery time. In this case, the greatest decrease in the recovery time for the test drug was observed at a dose of 294 mg/kg.

In the case of the Psilo-Balsam at doses of 35 mg/kg (48.6 %) and 18 mg/kg (44.3 %), a significant decrease in the recovery time is also observed. In this case, the

greatest decrease in the recovery time for the drug «Psilo-Balm» was observed at a dose of 35 mg/kg.

In the case of the drug “Dermoveit” at a dose of 1.75 mg/kg (44.3 %), a significant decrease in the recovery time was observed. However, at a dose of 0.88 mg/kg (5.7 %), although a decrease in recovery time was observed, it was not significant. At the same time, the largest reduction in the recovery time for the Dermoveit drug was observed at a dose of 1.75 mg/kg.

In the case of preparation «Zinc Ointment « at doses of 350 mg/kg (51.4 %) and 175 mg/kg (51.4 %) also observed a significant decrease in recovery terms. The greatest reduction in drug recovery terms «Zinc Ointment « is observed in both dosages of 350 mg/kg and 175 mg/kg.

If we compare the experimental breakdown by the reduction of the time of recovery the most effective doses of comparator drugs, the difference between them was not statistically reliable.

Table 2: The results of the study of antiallergic activity (by the timing of recovery)
($M \pm tm$; $p = 0.05$; $n = 6$)

Group	Result	% effect
The control	11.67 (9.95÷ 13.38)	-
“Fitosbor” (at a dose of 445.5 mg/kg)	6.50 (5.62÷ 7.38)	40.6 %
“Fitosbor” (at a dose of 294 mg/kg)	6.33 (5.25÷ 7.42)	48.7%
“Fitosbor” (at a dose of 220.5 mg/kg)	6.67 (4.71÷ 8.62)	45.9 %
“Psilo-Balm” (at a dose of 35 mg/kg)	6.00 (5.06÷ 6.94)	48.6 %
Psilo-Balm (at a dose of 18 mg/kg)	6.50 (5.40÷ 7.60)	44.3 %
Dermoveit (at a dose of 1.75 mg/kg)	6.50 (5.21÷ 7.79)	4 4.3 %
Dermoveit (at a dose of 0, 88 mg/kg)	11.00 (7.75÷ 14.25)	5.7 %
“Zinc ointment “ (at a dose of 35 0 mg/kg)	5.67 (5.12÷ 6.21)	51.4%
“Zinc ointment “ (at a dose of 175 mg/kg)	5.67 (5.12÷ 6.21)	51.4%

When assessing changes in the dynamics of an allergic reaction (inflammation), it was found (Table 3) that the test drug at doses of 445.5 mg/kg and 294 mg/kg already on the 3rd day of treatment, and at a dose of 220.5 mg/kg by 4 the day of treatment there was a significant decrease in the severity of the allergic reaction, which was traced to the end of treatment. In that case, the most pronounced decrease in the severity of the allergic reaction in the test drug was observed at a dose of 294 mg/kg.

In the case of the Psilo-Balsam preparation at doses of 35 mg/kg and 18 mg/kg, a significant decrease in the severity of the allergic reaction was observed already on the 4th and 3rd day of treatment, which can be traced to the end of treatment. Meanwhile, its most pronounced decrease in the severity of the allergic reaction in the Psilo-Balsam, which was observed at a dose of 18 mg/kg.

In the case of the drug « Dermoveit « at a dose of 1.75 mg/kg already on the 3rd day of treatment, there

was a significant decrease in the severity of the allergic reaction, which can be traced to the end of treatment. However, at a dose of 0.88 mg/kg, a significant decrease in the severity of an allergic reaction was observed only on the fifth day. In that case most of its marked reduction in severity of an allergic reaction in preparation « Dermoveyt » was observed at a dose of 1.75 mg/kg.

In the case of the drug “Zinc ointment” in doses of 350 mg/kg and 175 mg/kg already on the 4th and 3rd day of treatment, there was a significant decrease in the severity of the allergic reaction, which can be traced to the end of treatment. At the same time, its

most pronounced decrease in the severity of the allergic reaction in the drug « Zinc Ointment » was observed at a dose of 175 mg/kg.

If we compare the experimental data by the decrease of the dynamics of an allergic reaction (inflammation) of the most effective dose of the drugs under experiment and the most effective doses of comparator drugs, it reveals that the experimental data of the test drug and «Psili Balm» and « Dermoveyt » are comparable, and in the case the «Zinc ointment», there was no significant difference.

Table 3: The results of a study of changes in the dynamics of an allergic reaction (inflammation) (M±tm; p = 0.05; n = 6)

Days Treatment	The control	“Fitosbor” (at a dose of 445.5 mg/kg)	“Fitosbor” (at a dose of 294 mg/kg)	“Fitosbor” (at a dose of 220.5 mg/kg)
1 day (baseline)	5.00	4.67(4.12÷5.21)	5.00	5.00
2 day	5.00	4.67(4.12÷5.21)	5.00	5.00
3 day	5.00	4.33(3.79÷4.88)	4.33(3.79÷4.88)	4.50(3.93÷5.07)
4 day	5.00	2.67(1.81÷3.52)	2.33(1.25÷3.42)	2.83(1.03÷4.64)
5 day	5.00	1.83(0.80÷2.87)	1.33(0.25÷2.42)	1.67(0÷3.73)
6 day	4.67(4.12÷5.21)	0.67(0÷1.94)	0.50(0÷1.38)	1.17(0÷3.09)
7 day	3.83(2.80÷4.87)	0.33(0÷1.19)	0,17(0÷0.60)	0.67(0÷1.94)
8 day	2.83(1.44÷4.23)	0	0	0.50(0÷1.38)
9 day	2.00(0.67÷3.33)	-	-	0
10 day	1.33(0.06÷2.60)	-	-	-
11 day	0.83(0÷1.87)	-	-	-
12 day	0.33(0÷0.88)	-	-	-
13 day	0.17(0÷0.60)	-	-	-
14 day	0	-	-	-

Table 3: The results of a study of changes in the dynamics of an allergic reaction (inflammation) (M±tm; p=0.05; n = 6)

Days Treatment	“Psilo-Balsam” (dose of 35 mg/kg)	Psilo-Balm dose of 18 mg/kg)	Dermoveit (dose of 1.75 mg/kg)	Dermoveit (dose of 0.88 mg/kg)	“Zinc ointment” (dose of 350 mg/kg)	“Zinc ointment” (dose of 175 mg/kg)
1 day (ex. Comp.)	5.00	4.83(4.40÷5.26)	4.83(4.40÷5.26)	5.00	5.00	5.00
2 day	4.83(4.40÷5.26)	4.83(4.40÷5.26)	4.83(4.40÷5.26)	5.00	5.00	4.67(4.12÷5.21)
3 day	3.83(2.44÷5.26)	3.83(3.04÷4.62)	3.83(3.04÷4.62)	5.00	4.17(3.13÷5.20)	3.83(2.80÷4.87)
4 day	3,50(2,05÷4.95)	3.17(2.13÷4.20)	3.17(2.13÷4.20)	4.83(4.40÷5.26)	2.33(0.50÷4.17)	2,33(0.90÷3.77)
5 day	2.17(0.13÷4.20)	2.33(0.90÷3.77)	2.33(0.90÷3.77)	4.33(3.79÷4.88)	0.67(0.12÷1.21)	1.33(0÷2.91)
6 day	0.50(0÷1.38)	1.00(0÷2.76)	1.00(0÷2.76)	3.83(2.61÷5.06)	0	0

Days Treatment	“Psilo-Balsam” (dose of 35 mg/kg)	Psilo-Balm dose of 18 mg/kg)	Dermoveit (dose of 1.75 mg/kg)	Dermoveit (dose of 0.88 mg/kg)	“Zinc ointment” (dose of 350 mg/kg)	“Zinc ointment” (dose of 175 mg/kg)
7 day	0.17(0÷0.60)	0.50(0÷1.38)	0.50(0÷1.38)	2.83(0.80÷4.87)	-	-
8 day	0	0	0	2.67(0.40÷4.93)	-	-
9 day	-	-	-	2.33(0.27÷4.40)	-	-
10 day	-	-	-	1.67(0÷3.62)	-	-
11 day	-	-	-	1,50(0÷3,22)	-	-
12 day	-	-	-	1.17 (0÷2.56)	-	-
13 day	-	-	-	0.50 (0÷1.38)	-	-
14 day	-	-	-	0	-	-

Discussion

There are some data in the literature analyzing the effectiveness of antiallergic drugs. One interesting study analyzed the results of the treatment in forty-one patients⁷. Authors observed a significant difference between the acute radiation dermatitis scores on the test and the control arms⁸. Mometasone furoate cream reduced the stages of acute radiation dermatitis when the radiotherapy used over 6000 cGY⁹. Compared to the control arm, local application of mometasone furoate significantly reduced the singses of dermatitis like itching and pain regardless of the radiotherapy dose (Liao, 2019)¹⁰.

Bao et al conducted a trial treating one hundred and sixty-four cases of allergic rhinitis with the Chinese medicine Biminkang mixture. That mixture was mainly included the Kazak folk herb *Artemisia rupestris*. They compared the results of 52 cases of allergic rhinitis were treated with the patent medicine Biyankang. The results showed that Biminkang mixture was an effective drug in treating allergic rhinitis (Bao et al, 1997)¹¹.

However, another study concluded investigating the overall effects of herbal therapy against allergic pathologies concluded that there is an existence of a number of clinically important interactions, although cause and effect relationships have not always been established. Herbs and drugs may interact either pharmacokinetically or pharmacodynamically. Yet, for many of the interactions discussed here, the understanding of the mechanisms involved is incomplete. Taking herbal agents may represent a potential risk to patients under conventional pharmacotherapy (Izzo, 2005)³.

Based on the data obtained, we can conclude that the test drug has a reliable antiallergic activity, which is reliably manifested in the decrease of total allergic reaction, in a reduction in the recovery time and in a decrease of the dynamics of the severity of allergic reactions⁴. Also, the study of antiallergic activity showed that the tested drug has a comparable antiallergic activity with the reference and synthetic drugs, since the experimental pharmacological activity of the most effective doses of the drug, which we tested and the reference synthetic drugs have no statistically significant difference⁹.

Conclusion

The antiallergic activity of the phytocollection for the treatment of eczema in comparison with the reference drug was studied. According to the results of the study of antiallergic activity, the testing drug has a reliable antiallergic activity, which is comparable to reference synthetic drugs.

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