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Effect of *Delphinium staphisagria* in Murine Infection by *Myocoptes musculinus*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Biomedical investigations still rely on the use of laboratory animals, mice and rats are the most commonly used on experimentations. Past experimentations with these animals showed that they could be affected by environmental conditions and infections, causing interferences on researches. Colonies of mice and rats can be parasitized by ectoparasites. Ectoparasites may interfere with scientific researches, typically exacerbated when the animals are immunosuppressed, however, is not common to cause mortality on mice. The plant known as *Delphinium staphisagria* has been used for years on the treat of skin wounds caused by insects and other parasites such as scabies. The aim of this study is to evaluate the effectiveness of the plant *Delphinium staphisagria* on

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Myocoptes musculinus infections on mice (*Mus muscullus*), the experimental animal. The mice of the Parasitology Lab / DBS / UEM with four weeks of age, weighing approximately 28-30 g were evaluated for clinical diagnosis of infection *Myocoptes musculinus*. Once confirmed, the parasitic animals were divided into experimental groups consisting of 10 animals each as: (I) infected animals with *Myocoptes musculinus* untreated, (II) infected animals treated with *Delphinium staphisagria*, once daily added in drinking water, (III) animalsco-infected with strain of low lethality of *Trypanosoma cruzi* and treated with *Delphinium staphisagria*. (IV) animalsco-infected with low lethality strain of *T. cruzi* and untreated. The clinical and parasitological evaluation was conducted for 90 days. All animals treated with *Delphinium staphisagria* showed clinical and parasitological cure for the infection of *Myocoptes musculinus*.

Keywords: Staphisagria; ultradiluted; scabies; Trypanosoma cruzi.

1. INTRODUCTION

Biomedical investigations still rely on the use of laboratory animals, mice and rats are the most commonly used on experiments. Some researches on the past with these animals have showed that they can be affected by environmental conditions and infections, causing interference on researches. Colonies of mice and rats can be parasitized by ectoparasites (*Radfordia affinis*, *Myocoptes musculinus Myobia musculi*, *Radfordia ensifera*, *Poliplax spinulosa* and *Poliplax serrata*).

Ectoparasites may interfere on scientific researches, usually when they show exacerbate immunosuppression, however, is not common to cause mortality on mice [1,2,3,4]. *Myocoptes musculinus* is a common parasite on mice cited as having a cosmopolitan distribution and reported as common species on laboratory mice. It is called pseudo causes scabies that don't dig galleries, but causes a kind of waxing and flaking at the affected site.

The Chagas disease caused by *Trypanosoma cruzi* (*T. cruzi*) is characterized by intense inflammatory process with a strong component of generalized immunosuppression [5]. The *T. cruzi* is grouped into distinct strains circulating in both of the domestic cycle as the sylvatic cycle and is classified into Discrete Typing Units (DTUs). Parasites that belong to the DTU I develop parasitaemia that evolves strains with low multiplication rate reaching peak between late 20 to 30 days after infection, with a mortality rate of 50 days after infection.

The Colombian strain belongs to DTU I and was isolated from a human case that origins in Colombia. This strain has been used on experimental investigations since the beginning of Federici et al. [6] studies in a murine model. Experimental infection of mice with this strain results in the persistence of the population of *T. cruzi* parasite that induces a series of changes in the immune system with immunosuppression, polyclonal lymphocyte activations and intense general inflammatory process. These alterations may determine in animals an exacerbation of infection by *Myocoptes musculinus* [7].

The plant *Delphinium staphisagria*, has been used for years on the treat of skin wounds caused by insects and other parasites. It is an herbaceous plant *Ranunculaceae* family, reaching 1 to 1.5 meters tall, grown in shady places in France, Italy and southern Europe. The part of the plant used for preparation of the extract are the seeds. Each seed contains a

stone with a greasy substance with a strong odor that seems to be the active ingredient. Few studies have been conducted with this plant, and some studies show its composition rich in flavonoids oxodihydroatisine 19-22-O-acetyl-19-oxodihydroatisine and alkaloids azitine, dihydroatisine, delphinine, neoline, bullatineC (14-acetylneoline) chasmanine14-acetylchasmanine and atisinium chloride [8].

This study aims the evaluate of the effect of the *Delphinium staphisagria* ($1x10^{24}$) in murine infection by *Myocoptes musculinus* on animals with and without infection of the *Trypanosoma cruzi*.

2. MATERIALS AND METHODS

2.1 Animals

Male *Swiss Webster* mice, four weeks of age, weighing approximately 28-30 g, provided by the Central Animal Laboratory of the State University of Maringá, were used for the experiments. The protocol for the experiments was approved by the Ethics Committee of Animal Experiments 029/2011.

2.2 Clinical Evaluation

Animals kept in cages with food and water *ad libitum* were monitored daily for 30 days to evaluate the initial onset of clinical signs of disease development. Naturally infected animals that came from Central Animal Facility were selected for the study. The animals were kept in a vivarium of the Laboratory of Parasitology/DBS/UEM under ideal conditions of temperature 22ºC±2ºC, humidity 70% and photoperiod (light/dark cycle 12 h). The progression of the disease was evaluated, giving special attention to the macroscopic characteristics of the lesions and the intensity of the affected areas and itching.

2.3 Evaluation Parasitological

To detect mites on the mouse's skin were used techniques like skin scrapings, microscopic examination of crusts, tearing of hair and sticky tape. The material obtained was clarified and identified according to Noble & Noble [9] and Georgi [10].

2.3.1 Skin scrapings and microscopic examination of crusts

The skin of the mice was shaved with a scalpel blade blunt and round (# 10). The site selected for the scraping was with lesions (scabs) and parasite suspect. With the help of skin was shaved on opposite directions (backwards) until the capillary circulation has become evident. The collected fragments were disposed onto glass slides. The crusts were then digested in sodium hydroxide 10% then examined under the light of the microscope.

2.3.2 Hair plucking

Hairs were plucked with the aid of tweezers, and then placed on a glass slide and covered with mineral oil and immediately examined to search for the parasite.

2.3.3 Adhesive tape

The sticky side of the tape was put on affected area where there were injuries. The tape was then removed and glued to a glass slide, fragments of skin and mite's dust were attached to the blade allowing a microscopic examination.

2.4 Co-infection of Mice with *Trypanosoma cruzi* and *Myocoptes musculinus*

Infected mice by *Myocoptes musculinus* were infected with *Trypanosoma cruzi* Colombian strain. The number of parasites in the inoculum was determined according to the method of Brenner [11]. The inoculum was 10,000 tripomastigostas blood in 0.2 ml / animal.

2.5 Preparation of *Delphinium staphisagria*

The drug in the form of mother tincture, obtained from the laboratory HN CRISTIANO, São Paulo, Brazil, was diluted in $1x10^{24}$ alcohol/ water 8%. The method used to prepare the drug was described in the Brazilian Homeopathic Pharmacopoeia [12,13]. This dilution was considered absent toxicity

2.6 Treatment Schedule

Considered 10 animals per group of experimentation: (I) untreated *Myocoptes musculinus* infected animals, (II) *Myocoptes musculinus* infected animals treated with *Delphinium staphisagria* diluted in $1x10^{24}$ alcohol/ water 8%, once daily added to the drinking water (1:10 ml) available *ad libitum,* (III) animals co-infected with *T. cruzi* Colombian strain treated with *Delphinium staphisagria* diluted in $1x10^{24}$ alcohol/ water 8% once daily added to the drinking water (1:10 ml) (IV) animals co-infected with *T. cruzi* Colombian strain and untreated. The experimental groups were treated for 60 days being clinically and parasitologically evaluated every 3 days.

2.7 Statistical Analysis

Statistics comparing groups were performed using the program GraphPad Prism (GraphPad, San Diego, CA, USA) using the Student t test. P values <0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1 shows the result of injuries and parasitism on experimental groups with the respective treatment time of 30 consecutive days.

Table 1. Effect of *Delphinium staphisagria* in murine infection by *Myocoptes musculinus* considering parasitological parameters of infection

Experimental group	Lesion	Parasites/eggs
Group I	6	12/16
Group II	1*	0/0*
Group III	4*	1/0*
Group IV	largesizes (impossiblenumber)	

Each value represents the mean *Myocoptes musculinus* infected mice: Group I untreated, Group II treated with *Delphinium staphisagria* diluted in $1x10^{24}$ alcohol/ water 8%.once daily

added to the drinking water (1:10 ml) Group III animals co-infected with *T. cruzi* Colombian strain treated with *Delphinium staphisagria* diluted in $1x10^{24}$ alcohol/ water 8% once daily added to the drinking water (1:10 ml) (IV) animals co-infected with *T. cruzi* Colombian strain and untreated. The treatment began after confirmed infection and was continued for consecutive 30 days. *P < .05 (ANOVA, Tukey's test)

These results show that in groups where *D. staphisagria* was administered significant reduction in the number of injuries caused by parasites and the number of parasites and eggs occurred. It is evident also that animals coinfected with *T. cruzi* have exacerbated lesions and number of parasites/eggs. In the coinfected group and treated with *D. staphisagria* (Group III) significant reduction in lesions and reduction in the number of parasites/eggs occurred. These results strongly suggest that *D. staphisagria* has a direct effect on murine infection by *Myocoptes musculinus* drastically reducing the infestation is in animals coinfected with *T. cruzi* or not.

Mice naturally infected with *Myocoptes musculinus* are not rare in experimental animal rooms and depending on the degree of health of the animal house can become a serious problem in the experimental groups. Infestations with *Myocoptes musculinus* can lead to animal health problems and may impose unwanted research variables by affecting the immune and physiologic functions of mice. Our work highlights a situation of natural infection of animals. This fact was being examined at the time when the work was proposed and carried out as an attempt to novel therapies to approach this problem since few drugs are available for this treatment and have a relative toxicity. The experiments performed with *Delphinium staphisagria* diluted in $1x10^{24}$ alcohol/ water 8%.once daily added to the drinking water (1:10 ml) administered *ad libitum* showed a significant improvement in the infected animals. Progressive reduction in the number of lesions and parasites occurred until complete clinical and parasitological cure. Numerous treatment protocols have been described with the aim of controlling the infestation *Myocoptes musculinus* but most of these have shown toxicity to some strains of mice and young animals [14]. Treatment and eradication of skin mites on mice has been a little studied [15,16] limited to certain drugs extremely toxic and that should be used for short time. Gressler et al. [17] demonstrated the efficacy of ivermectin in the treatment of mice infected with mites.

Our work on the other hand shows that *D. staphisagria* not only induces aclinical and parasitological improvement in mice and is presented free of toxicity since it was administered *ad libitum* for 60 days without toxicity in mice not occurring animal mortality.

Fig. 1 shows the results of lesions caused for *Myocoptes musculinus* on animals co-infected with *T. cruzi* Colombian strain after 30 and 60 days of treatment with *Delphinium staphisagria*.

Animals co-infected with *T. cruzi* (Group III and IV) showed a worse outcome of the infection *Myocoptes musculinus* greater extent and depth of the lesions (Fig. 1 - A). This fact can be explained due to an immunosuppression caused by infection with *T. cruzi* and an exacerbating infection of *Myocoptes musculinus* could be also a gateway to secondary infections.

Mice with sub-clinical infection by *Myocoptes musculinus* once infected with other micro-organisms with specific experiments such as *T. cruzi* showed an exacerbated immune impairment from the infection and manifested by larger and deeper lesions (Group IV) although the quantity of parasites found was not statistically different (Group I).

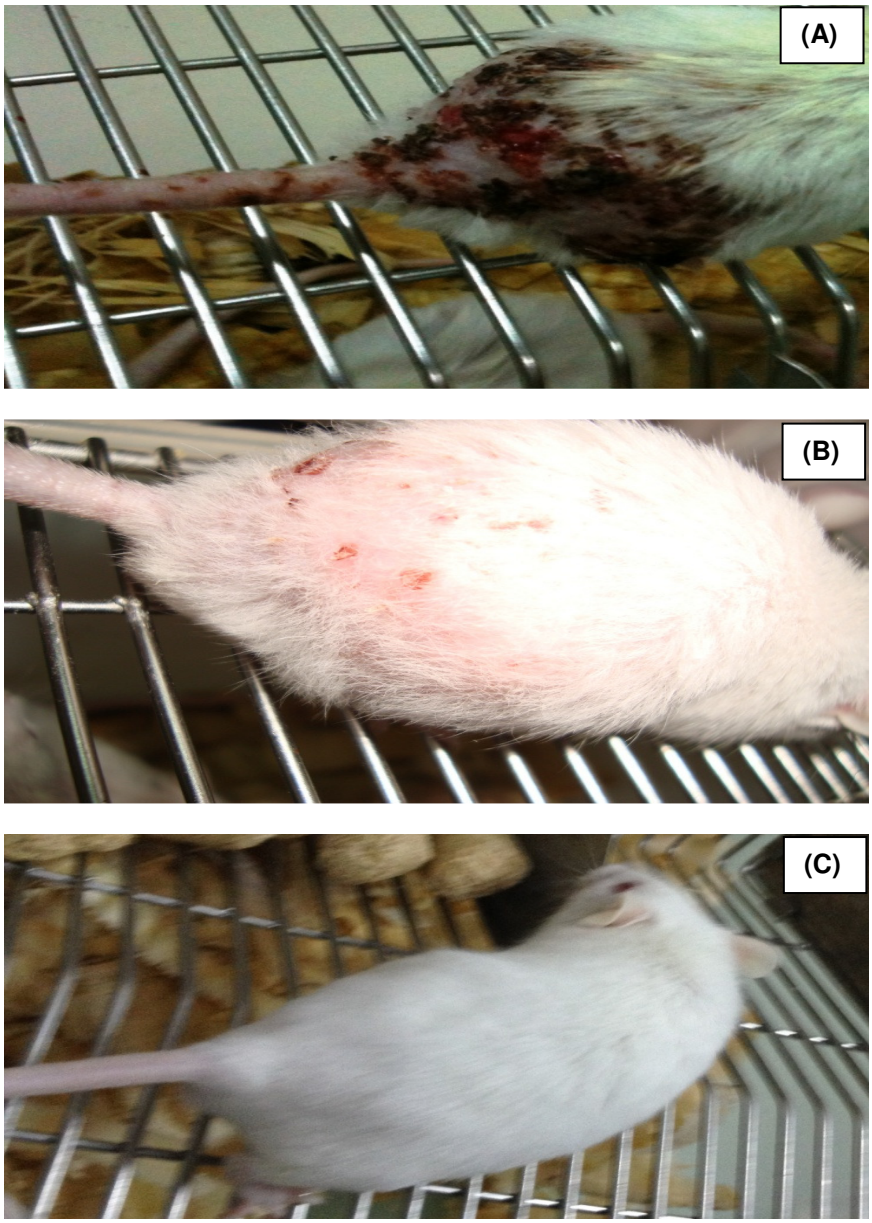

Fig. 1. Effect of *Delphinium staphisagria* in murine infection by *Myocoptes musculinus* coinfected with *Trypanosoma Cruzi*: aspects of the lesions

(A) Animals infected with Myocoptes musculinus and Trypanosoma cruzi untreated; (B) Animals infected with Myocoptes musculinus and Trypanosoma cruzi treated with Delphinium staphisagria diluted in $1x10^{24}$ alcohol/ water 8%.once daily added to the drinking water (1:10 ml) 30 days (C) Animals infected with Myocoptes musculinus and Trypanosoma cruzi treated with Delphinium staphisagria diluted in $1x10^{24}$ alcohol/ water 8%.once daily added to the drinking water (1:10 ml) 60 days

In vitro and *in vivo* trypanocidal activities of nine flavonoids isolated from the aerial parts of *Delphinium staphisagria* were studied in acute and chronic phases of Chagas' disease. The

antiproliferative activity of these compounds against *T. cruzi* (amastigotes, epimastigotes and trypomastigotes), in some cases antitripanosomatida exhibited more potent activity and less toxicity than the reference drug, benznidazole [18]. In vitro analysis with ultra structural studies of excretion of metabolism, were also performed to identify the possible mechanisms of action of the compounds tested. Changes, especially at mitochondrial level could explain the metabolic changes in the production of acetate and succinate, perhaps due to the disturbance of the enzymes involved in sugar metabolism inside the mitochondria [19]. No signs of toxicity were detected on mice treated with the flavonoids tested the parasitic load was significantly lower than on the control group treated with benznidazole.

According to these studies *Delphinium staphisagria* would be contributing to the positive development of *Myocoptes musculinus* infection on mice co-infected with *T. cruzi* by promoting the decrease of parasitism by *T. cruzi* which would be leading to a decrease in inflammation and subsequent immune response that would best as clinical improvement in lesions caused by *Myocoptes musculinus* (group III).

It is observed that Group II was not coinfected and also demonstrate an improvement in clinical infection *Myocoptes musculinus* demonstrating that *Delphinium staphisagria* could be stimulating a clinical and parasitological cure since there were not parasites and live eggs lesions (Table 1). It is observed that Group I was not coinfected and also demonstrate an improvement in clinical infection *Myocoptes musculinus* demonstrating that *Delphinium staphisagria* could be stimulating a clinical and parasitological cure since there were parasites and live eggs lesions (Table 1).

A lot of studies examining the action of substances ultradiluted demonstrate leukocyte migration and activation of the macrophages in vivo and in vitro [20,21,22]. The study of Wagner et al. [23] demonstrates experimentally the effect of low doses of cell extracts of plants and demonstrated the effect observed in the dose-response curve. This group of study reports that high concentrations (100 ng-10 mg/ml) of naphthoquinones, and cytostatic agents (vincristine, methotrexate, and fluorouracil) inhibit the transformation of lymphoid and granulocyte phagocytosis while low concentrations (10 pg, 10 fg / ml) have a stimulatory effect and intermediate doses have no effect. The authors suggest that the antitumor effects of the plant extracts could be explained by its mechanism of double-effect dose. This phenomenon was also described on toxicology as the law of Arndt-Schultz [24] whose theory was developed as an effect "hormesis" [25,26].

Although the study can not assert a complete cure of animals infested with *M. musculinus* since it would be necessary evaluation more sensitive diagnostic methods such as PCR our results obtained with *Delphinium staphisagria* shown on the beneficial effect promoting improvement on inflammatory and immune response with consequent clinical improvement of animals.

The results obtained with diluted *Delphinium staphisagria* $1x10^{24}$ could be extrapolated to others animals species infected by mites and further studies may be conducted in order to elucidate this effect.

4. CONCLUSION

This study provides evidence that *Delphinium staphisagria* diluted in $1x10^{24}$ alcohol/ water 8% administered *ad libitum* causes a significant improvement in the infected animals by

Myocoptes musculinus showed a progressive reduction in the number of injuries caused by parasite.

CONSENT

Not applicable.

ETHICAL APPROVAL

Ethical clearance was sought for and obtained from Ethics Committee of Animal Experiments- State University of Maringá, Brazil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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