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Therapeutic and pathogenetic animal models for *Dolichos pruriens*

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The therapeutic and pathogenetic effects of *Dolichos pruriens* were evaluated using experimental models in rats. In the therapeutic experiment Wistar rats were housed in a heated environment (25 ± 3 °C) to induce itch, and treated with ascending potencies *D. pruriens* (6 cH, 9 cH, 12 cH and 30 cH), each for 10 days. The positive control group received vehicle (ethanol 30% in water). The negative control group received no treatment and were kept at a standard temperature.
In the pathogenetic experiment, all animals were kept at a temperature of 20 ± 3 °C and treated for 30 consecutive days with *D. pruriens* 6 or 30 cH, or ethanol vehicle, or no treatment. The experiments were performed blind.
The statistical analysis used Bartlett's test, followed by ANOVA/Tuckey–Krammer or Kruskal–Wallis/Dunn. The results point to the existence of therapeutic effects, with inhibition of the itching, skin lesions and fur thinning produced by heat, more evident in later observations, with the 9 12, and 30 cH potencies (Kruskal–Wallis/Dunn; $P = 0.001$). No changes were observed in the other parameters, such as open field activity and laterality of the itching. In the pathogenetic experiment, no changes were observed in any parameters examined. We conclude that the proposed experimental model demonstrates the therapeutic effect of *D. pruriens*, but not its pathogenetic effects. *Homeopathy* (2006) **95**, 136–143.

Keywords: homeopathy; *Dolichos pruriens*; animal model; itch; open field; grooming; pathogenesis

Introduction

The therapeutic and pathogenetic effects of *Dolichos pruriens* were evaluated using experimental models[1–12] in rats. *Mucuna pruriens* or *D. pruriens* is a leguminous creeper,[13–20] native to tropical regions of Asia, the Americas and Africa.[21] The pods are thick and leathery, covered by rough reddish hairs that are easily released, causing intense skin itching.[20] The mother tincture is prepared from the dry hairs.[22] *D. pruriens* prepared according to the homeopathic technique can induce symptoms in healthy humans, as described in several *materia medica*. Its main action is an intense skin itch, with no visible lesion; the symptoms worsen when the patient is lying down, at night, and with heat. Other less common symptoms described include senile itch worse on the right side of the body, nervous hypersensitivity and restlessness, muscular shaking, chronic spasms of the limbs, increased sensitivity of the gums, sore throat worse on swallowing, constipation, distended abdomen, right ear pain, bilious vomiting and jaundice.[13,19,22]

This information has been obtained from healthy humans, but pathogenetic studies in animals could yield more specific information that may be useful in

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the clinical veterinary practice. Such studies can also be useful when it is not possible to carry these out in humans, for example due to ethical issues or to avoid the placebo effect.

The aim of the present study is to verify whether Open field and Grooming, two behavioural experimental models, can be used as instruments for developing knowledge about veterinary homeopathic use of *D. pruriens* and for observing its pathogenetic effect.

Material and methods

Preparation of *D. pruriens*

Homeopathic *D. pruriens* was prepared according to the Hahnemannian method. To prepare the 6cH potency six serial 1:100 dilutions were made from the mother tincture in aqueous alcohol solution (30% alcohol), with 100 succussions for each dilution. The same procedure was used for all potencies. The medicine was prepared by the Homeopathic Manipulation Pharmacy "Núcleo da Manipulação" (São Paulo), authorized by ANVISA (Brazilian Sanitary Vigilance Agency). The mother tincture was obtained from DHU, Germany, batch ES87721, expiry date 04/2006. All experiments were performed in 2003 and 2004. To avoid labelling errors, three different professionals participated in the preparation and coding, the codes remaining blind until the end of the experiment.

Animals

Adult male Wistar rats weighing 180–300 g were used, obtained from the UNITOX-UNISA rodent facility (Laboratório Universitário de Análises Toxicológicas—UNISA, Brazil). They were kept in plastic cages (5 animals per cage), under controlled temperature (25 ± 3 °C or 20 ± 3 °C) and light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.) with food and water ad libitum. The animals were handled according to the International Guide Principles for Biomedical Research Involving Animals (CIOMS)—Geneva, 1985[23] (Figure 1).

Therapeutic model

The rats (15 animals/group) were kept in a heated environment (25 ± 3 °C) and treated with *D. pruriens* in an ascending scale of potency—6 cH, 9 cH, 12 cH and 30 cH, on a daily basis in the drinking water (1 ml of *D. pruriens* was diluted into 499 ml of drinking water). Each potency was administered for ten consecutive days, followed by behavioural observation. The positive control group received 30% aqueous alcohol solution, diluted 1:500 in their drinking water for the same period. The ascending scale of potency was chosen to mimic clinical conditions. A temperature of 25 ± 3 °C was controlled by a thermo-hygrometer instrument checked daily and maintained at this baseline. The negative control group received no treatment and was kept at 20 ± 3 °C. At 10 days intervals, behavioural sessions were carried out for each animal, four sessions in total.

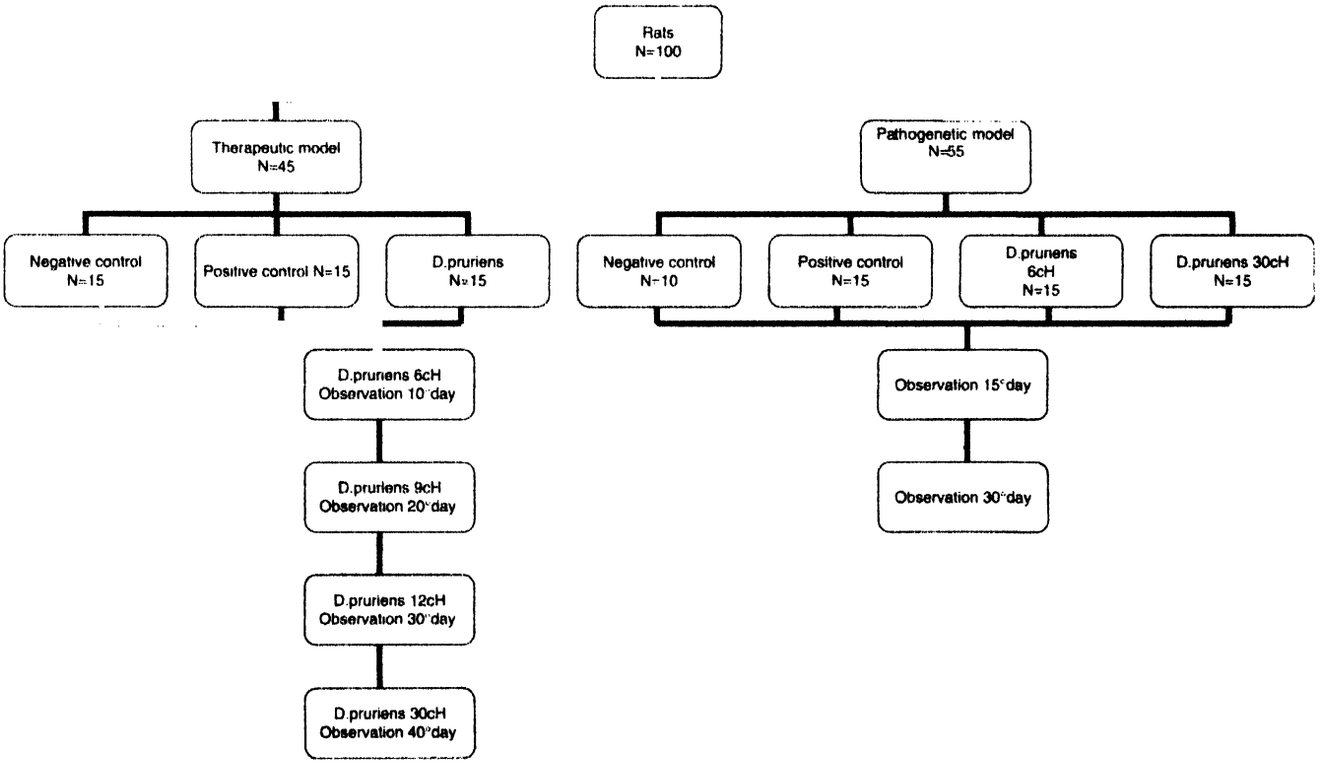

Figure 1 Flow chart of experiment.

Proving model

Two groups of Wistar rats were treated with *D. pruriens* 6cH and 30cH (15 animals/group), in the drinking water (1 ml of *D. pruriens* in 100 ml of water, 1 ml of this solution added to 499 ml of drinking water). The positive control group received 30% aqueous alcohol solution, in the same manner, for the same period. The negative control group (10 animals/group) received no treatment. The room temperature was maintained at 20 ± 3 °C and the positions of cages were changed periodically, to ensure that any effects observed could be exclusively ascribed to the homeopathic treatment. After 15 and 30 days of treatment, behavioural observations were carried out on each animal.

Behavioural sessions

Grooming was observed, noting its laterality, for 4 min in the therapeutic model and 5 min in the proving model, to quantify itch behaviour. Five minutes open field sessions were carried out to measure ambulation, rearing, grooming, freezing and defecation. The animals were observed first in the open field then for grooming behaviour. All animals were handled every day at 6:30 a.m. and 11:30 a.m., to observe any hair loss or skin lesions, scored according to previous studies[25] (Table 1). At the end of the experiment, all animals were subjected to necropsy and the incidence of lesions was recorded for each group.

Statistical analysis

For the therapeutic model, Bartlett's test was used to test the normality of the distribution of the behavioural variables studied. ANOVA Tuckey–Kramer or Kruskal–Wallis/Dunn was used according to the distribution. The incidence of macroscopic lesions was analysed by the Fisher test. In the pathogenetic model, simple multiple comparisons between means and standard deviations may introduce bias, if only a few animals are sensitive. The number of animals showing behavioural scores more than two standard deviations from the mean of the respective group was therefore also computed. The observed frequency of these animals in the experimental and control groups was analysed by the Fisher test. In addition, a "delta value" of behaviour scores was calculated by subtracting the value measured on the 15th day from that measured on the 30th day, to detect the possible temporal variations in the occurrence of pathogenetic symptoms. These data were analysed by ANOVA followed by Tuckey–Kramer test. In all cases, the level of significance was set at $P \leq 0.05$.

Table 1 Skin scoring system

Fur thinning scores
(–) = Score 0 = no thinning
(+) = Score 1 = mild thinning
(++) = Score 2 = moderate thinning
(+++) = Score 3 = severe thinning

Skin lesion scores
(–) = Score 0 = Absent
(+) = Score 1 = Discrete erythema with some papules
(++) = Score 2 = Erythema, papules and delineated crust
(+++) = Score 3 = Intense erythema, papules and multifocal crusts

Results

Therapeutic model

The data obtained from the open field experiments showed that the positive control animals treated with aqueous alcohol solution demonstrated a significant increase in grooming behaviour (Kruskal–Wallis, $P<0.05$) compared to the negative control group and to the experimental group treated with *D. pruriens*. The differences between groups are shown in Table 2. Concerning the grooming parameters, high levels of itch behaviour were observed (Kruskal–Wallis/Dunn; $P<0.05$) in the positive control group compared to negative control, while the *D. pruriens* treated group showed a protective action on this parameter (Table 3).

The onset of itch behaviour and fur thinning was also delayed in the *D. pruriens* treated group. The *D. pruriens* treated animals began to show lesions about the 34–36 days. The positive control group, kept at the same temperature began to show lesions on the 20th day, treated animals showed milder lesions than the control group (Table 4).

An increase in water consumption, was observed in the positive control group compared to *D. pruriens* treated group. Food consumption in the negative control group was higher than in animals exposed to high temperatures. But animals treated with *D. pruriens* consumed more food than positive control, suggesting better adaptation to high temperatures (Table 5).

Pathogenetic model

Open field data (grooming, ambulation, rearing, freezing) did not show differences between groups. The animals showed neither itch nor hair loss (Tables 6 and 7). The group treated with *D. pruriens* 6CH consumed more water than the other groups, and food consumption of the *D. pruriens* 6cH-treated animals was higher than the *D. pruriens* 30cH-treated animals (Table 8).

No significant differences were observed in either weight gain nor in the "delta value" analysis (Table 9) The frequency of animals showing clear pathogenetic symptoms was not significant (Table 10). No relevant macroscopic lesions were found.

Discussion

The results suggest that *D. pruriens* in increasing potencies protects animals from itching, as detected by observation of grooming behaviour. *D. pruriens* was

Table 2 Results of open field test (mean ± standard deviation)

Parameters	*1st observation*	*2nd observation*	*3rd observation*	*4th observation*
Grooming				
Negative control	3.8 ± 2.75	1.86 ± 1.18*	1.6 ± 1.1	2.13 ± 1.50
Positive control	4.8 ± 2.79	4.60 ± 2.53	6.2 ± 3.8*	6.33 ± 2.92**
D. pruriens potencies	4.0 ± 2.45	3.66 ± 2.19	2.6 ± 2.2	2.93 ± 2.65
Locomotion				
Negative control	56.26 ± 17.12	48.66 ± 22.72	45.0 ± 21.7***	35.93 ± 16.90
Positive control	53.46 ± 10.06	45.40 ± 19.81	24.20 ± 17.5	36.66 ± 24.15
D. pruriens potencies	45.53 ± 15.83	49.60 ± 21.74	21.1 ± 16.6	30.33 ± 26.02
Rearing				
Negative control	12.80 ± 5.38	8.20 ± 5.30	5.73 ± 3.3***	6.00 ± 2.67
Positive control	10.53 ± 7.23	8.06 ± 6.00	2.6 ± 2.9	6.06 ± 6.18
D. pruriens potencies	10.20 ± 4.36	7.53 ± 5.02	2.1 ± 2.9	4.33 ± 4.59
Defecation				
Negative control	5.06 ± 2.54	4.86 ± 2.20	4.6 ± 2.6	3.8 ± 1.82
Positive control	4.26 ± 2.18	3.20 ± 3.27	5.5 ± 2.2	3.2 ± 3.05
D. pruriens potencies	4.93 ± 2.68	2.60 ± 2.94	4.1 ± 2.8	3.4 ± 3.13

Each animal was observed for 4 min.
*Kruskal–Wallis/Dunn, $P < 0.05$.
**Kruskal–Wallis/Dunn, $P < 0.01$.
***ANOVA/Tukey–Krammer, $P < 0.05$ relative to the other groups.

Table 3 Grooming laterality in the therapeutic model: time, in minutes, of grooming behaviour (mean ± standard deviation)

Parameters	*1st observation*	*2nd observation*	*3rd observation*	*4th observation*
Right				
Negative control	0.14 ± 0.14	0.05 ± 0.1	0.1 ± 0.2	0.09 ± 0.11
Positive control	0.16 ± 0.14	0.17 ± 0.15*	0.59 ± 0.5*	0.64 ± 0.5**
D. pruriens potencies	0.24 ± 0.21	0.21 ± 0.3	0.4 ± 0.4	0.16 ± 0.20
Left				
Negative control	0.14 ± 0.13	0.05 ± 0.1	0.1 ± 0.2	0.09 ± 0.11
Positive control	0.13 ± 0.11	0.18 ± 0.16*	0.62 ± 0.5***	0.62 ± 0.52†
D. pruriens potencies	0.25 ± 0.21	0.2 ± 0.29	0.2 ± 0.2	0.18 ± 0.23

Each animal was observed for 4 min.
*Kruskal–Wallis/Dunn; $P < 0.05$ relative to negative control.
**Kruskal–Wallis/Dunn; $P < 0.01$ relative to the other groups.
***Kruskal–Wallis/Dunn; $P < 0.01$ relative to negative control.
†Kruskal–Wallis/Dunn; $P < 0.05$ relative to the other groups.

used in potencies of 6 and 30 cH (6 cH has a composition close to the limits of measurement of the substance and 30 cH is clearly beyond Avogadro's number) to verify its possible pathogenetic effect,[26,28] but this could not be demonstrated.

The main symptom of *D. pruriens* is the intense itching, worse at night and with heat.[13,15,17,19,22] This symptom is similar to that observed in the present experiment, where rats treated with *D. pruriens* were subjected to a heated environment. Data obtained from the open field test showed that positive control group showed more itching behaviour than the animals not subjected to heat (control group), the animals treated with *D. pruriens* showed itch behaviour levels comparable to the control group, with no alterations of laterality. It could therefore be hypothesised that the treatment prevented reactions induced by heat, this was more evident after some weeks of treatment, as a time and potency-dependent phenomenon. This pattern of effect is often described in homeopathic treatments,[29] particularly in patients who develop itching secondary to haemodialysis.[30]

This could be considered a true *similia* model, since the itching behaviour induced by heat is modulated by particular mechanisms (dilatation of vessels and increase in sebaceous glandular activity, leading to increased auto-cleaning behaviour), that differ from the acute dermatitis with histamine release, as is triggered by contact with *Dolichos* pods.

The appearance of itching behaviour can be explained by the stress of the heat,[24] or by suppression of the immune system[31] which can lead to various forms of dermatitis.[24,32,33] The putative effects of *D. pruriens* upon stress and immune system therefore deserves further study.[34,37]

Water consumption by rats varies according to the temperature.[24] In this experiment, there was a statistically significant increase (ANOVA, $P < 0.01$) in water

Table 4 Skin lesions and fur thinning scores (mean ± standard deviation) in different groups, from the 20th to the 41st day of observation

Observation day	*Negative control*		*Positive control*		*D. pruriens potencies*	
	Lesion	*Fur thinning*	*Lesion*	*Fur thinning*	*Lesion*	*Fur thinning*
20	0.0 ± 0.0	0.0 ± 0.0	0.16 ± 0.46*,†	0.13 ± 0.35	0.0 ± 0.0	0.0 ± 0.0
21	0.0 ± 0.0	0.0 ± 0.0	0.36 ± 0.76*,†	0.13 ± 0.35	0.0 ± 0.0	0.0 ± 0.0
22	0.0 ± 0.0	0.0 ± 0.0	0.43 ± 0.77**,‡	0.13 ± 0.35	0.0 ± 0.0	0.0 ± 0.0
23	0.0 ± 0.0	0.0 ± 0.0	0.73 ± 1.17**,‡	0.13 ± 0.35	0.0 ± 0.0	0.0 ± 0.0
24	0.06 ± 0.36	0.0 ± 0.0	0.66 ± 1.06*	0.13 ± 0.35	0.26 ± 0.69	0.0 ± 0.0
25	0.06 ± 0.36	0.0 ± 0.0	0.73 ± 1.23*	0.13 ± 0.35	0.46 ± 0.86	0.0 ± 0.0
26	0.06 ± 0.36	0.0 ± 0.0	0.66 ± 1.12*	0.13 ± 0.35	0.30 ± 0.59	0.0 ± 0.0
27	0.06 ± 0.36	0.0 ± 0.0	1.13 ± 0.97**,†	0.13 ± 0.35	0.53 ± 0.62*	0.0 ± 0.0
28	0.06 ± 0.25	0.0 ± 0.0	1.16 ± 1.02**,†	0.13 ± 0.35	0.63 ± 0.88*	0.0 ± 0.0
29	1.06 ± 0.98	0.0 ± 0.0	1.1 ± 0.98	0.26 ± 0.79	0.70 ± 0.91	0.0 ± 0.0
30	0.0 ± 0.0	0.0 ± 0.0***	1.06 ± 0.86**,†	0.80 ± 0.94	0.05 ± 0.62*	0.26 ± 0.45
31	0.0 ± 0.0	0.0 ± 0.0***	1.00 ± 0.78**,†	0.73 ± 0.79	0.05 ± 0.62*	0.26 ± 0.45
32	0.0 ± 0.0	0.0 ± 0.0***	1.03 ± 0.76**,†	0.73 ± 0.79	0.05 ± 0.62*	0.26 ± 0.45
33	0.0 ± 0.0	0.0 ± 0.0***	1.00 ± 0.78**,†	0.73 ± 0.79	0.05 ± 0.62*	0.26 ± 0.45
34	0.0 ± 0.0	0.0 ± 0.0***	1.00 ± 0.78**,†	0.80 ± 0.86	0.05 ± 0.62*	0.26 ± 0.45
35	0.0 ± 0.0	0.0 ± 0.0***	1.00 ± 0.78**,†	0.80 ± 0.86	0.05 ± 0.62*	0.26 ± 0.45
36	0.0 ± 0.0	0.0 ± 0.0***	0.2 ± 0.55*,†	1.00 ± 0.75‡	0.0 ± 0.0	0.26 ± 0.45
37	0.0 ± 0.0	0.0 ± 0.0***	0.0 ± 0.0	0.93 ± 0.59‡	0.0 ± 0.0	0.26 ± 0.45
38	0.0 ± 0.0	0.0 ± 0.0***	0.0 ± 0.0	0.93 ± 0.59‡	0.0 ± 0.0	0.26 ± 0.45
39	0.0 ± 0.0	0.0 ± 0.0***	0.0 ± 0.0	0.93 ± 0.59‡	0.0 ± 0.0	0.26 ± 0.45
40	0.0 ± 0.0	0.0 ± 0.0***	0.0 ± 0.0	0.86 ± 0.74	0.0 ± 0.0	0.33 ± 0.48
41	0.0 ± 0.0	0.0 ± 0.0***	0.0 ± 0.0	0.86 ± 0.74	0.0 ± 0.0	0.33 ± 0.48

From the 1st to the 19th day there were no lesions and these days are not shown.
*Kruskal–Wallis/Dunn; $P<0.05$ relative to negative control.
**Kruskal–Wallis/Dunn; $P<0.001$ relative to negative control.
***Kruskal–Wallis/Dunn; $P<0.01$ relative to positive control.
†Kruskal–Wallis/Dunn; $P<0.05$ relative to *D. pruriens* ascending potencies.
‡Kruskal–Wallis/Dunn; $P<0.001$ relative to *D. pruriens* ascending potencies.

Table 5 Water (ml) and food (g) consumption in the therapeutic model

	Negative control	*Positive control*	*D. pruriens potencies*
Food consumption	103.60 ± 10.29	96.94 ± 8.74*	98.96 ± 9.24
Water consumption	137.96 ± 16.49	146.90 ± 21.44	131.59 ± 15.45**

The values represent mean ± standard deviation of daily consumption.
*ANOVA, Tukey–Kramer; $P<0.01$ relative to the negative control.
**ANOVA, Tukey–Kramer; $P<0.01$ relative to the aqueous alcohol-treated animals.

consumption in the positive control group (treated with aqueous alcohol solution) compared to the group treated with *D. pruriens*. On the other hand, food consumption by the animals not exposed to heat was greater than that of the other groups, but statistically significant (ANOVA, $P<0.01$) only when compared to positive control group. So, the animals treated with *D. pruriens* ate better, even when exposed to heat. According to Lopes *et al*[38] food consumption is modulated by internal and external factors and it is known that stress can reduce food consumption, depending on the nature of the stressing agent (in this case, heat). Considering the relation between heat and stress, the hypothesis that *D. pruriens* treatment could prevent stress discomfort is again supported.

Regarding body weight, although the ANOVA did not show significant differences between groups, there was a trend to weight gain in animals treated with *D. pruriens*, compared to the positive control. Other studies have also shown the effect of a high room temperature on gain in body weight. Oliveira *et al*[39] demonstrated that chickens exposed to high temperatures showed reduced weight gain. Vieira[40] also observed these effects.

Unlike the animals exposed to 25 °C, skin crusts were not observed in the proving experiment. This symptom is compatible with the descriptions obtained from the *D. pruriens materia medica* as a "non-lesion itch".[13,15,17,19]

Precautions were taken to ensure the reliability of the experiment: the open field observations, grooming and daily examination of the animals were all performed in the morning, between 6:30 a.m. and 11:30 a.m., the best time to observe any itch increase in rats (sleeping time). This constant rhythm also ensured the absence of any interference by the circadian cycle on the results. According to Majerowicz,[41] the regularity of this cycle is very important for the maintenance of behavioural normality of the animals. Automatic control of the light–dark period throughout

the pathogenetic and therapeutic experiments also helped to maintain this steady state.

The presence of grooming has been described as an indicator of emotional responsiveness, stress and behaviour alteration.[8-11,42] Some substances induce or increase grooming, such as ACTH,[43,44] LHRH,[45,46] Panalox notoginseng[9] and prolactin.[11] In this study, the grooming model was modified to allow the evaluation of itch in the animals.[47] Such modifications have been used in other studies to provide an objective parameter to be measured in laboratory studies. For itching in the therapeutic model, four observations, repeated every 10 days, were necessary. The possibility of habituation phenomena occurring in the open field was not considered because, according to Masur and Martz,[48] this occurs only after the 6th open field session and when the interval between trials is below 48 h.

From a more general perspective, this study could be very pertinent to veterinary clinical practice,[49] because of the many animals (mainly dogs and cats) that suffer from chronic skin problems, itching lesions or heat sensitivity, and the lack of effective long-term therapy.

However, considering the peculiarities of homeopathy, the difficulties of defining a well-delineated experimental model in order to show pathogenetic effects remains a major challenge. This is because of the requirement of selecting individual animals which are susceptible to a certain medicine, and also because part of the homeopathic pathogenetic observation is of subjective features which are often masked in animal models.[50,51]

Mahé,[27] for example, in a rabbit pathogenetic model, did not observe statistically significant differences in the total number of new symptoms between *Arsenicum album* treated group and placebo; however in the treated group, some individuals presented many pre-existing symptoms. Also, sometimes, the pathogenetic effects are observed only accidentally. For example, the use of *Kreosotum* in an experiment carried out for the prevention of dental caries in rats produced pathogenetic effects (hair loss) in about 40% of the treated animals.[12]

A systematic review of pathogenetic homeopathic trials performed between 1945 and 1995,[52] showed that

Table 6 Results of open field of rats treated or not with *D. pruriens* 6 CH or 30 CH (mean ± standard deviation) pathogenetic model

	1st observation	*2nd observation*
Grooming		
Negative control	2.7 ± 1.70	2.6 ± 1.35
Positive control	3.2 ± 2.11	2.87 ± 2.56
D. pruriens 30 CH	3.0 ± 1.73	2.47 ± 2.2
D. pruriens 6 CH	3.0 ± 2.1	3.2 ± 2.51
Locomotion		
Negative control	57.5 ± 17.56	40.2+20.34
Positive control	60.6 ± 19.32	43.4 ± 21.94
D. pruriens 30 CH	59.06 ± 15.41	41.4 ± 29.72
D. pruriens 6 CH	57.8 ± 22.01	49.33 ± 33.23
Rearing		
Negative control	7.7 ± 3.02	4.2 ± 3.85
Positive control	8.4 ± 4.78	5.33 ± 3.66
D. pruriens 30 CH	6.27 ± 4.45	3.6 + 4.94
D. pruriens 6 CH	7.4 + 7.38	5.6 ± 5.45
Defecation		
Negative control	4.9 ± 2.18	4.9 ± 2.42
Positive control	5.07 ± 3.2	3.93 ± 3.51
D. pruriens 30 CH	6.07 ± 2.52	4.07 ± 3.1
D. pruriens 6 CH	5.4 ± 3.11	5.27 ± 3.24
Freezing		
Negative control	0.12 ± 0.38	0.29 ± 0.76
Positive control	0.06 ± 0.16	0.3 ± 0.9
D. pruriens 30 CH	0.10 ± 0.17	0.57 ± 0.84
D. pruriens 6 CH	0.2 ± 0.15	0.08 ± 0.17

Each animal was observed for 5 min. ANOVA.

Table 7 Grooming laterality, proving model: time, in minutes, of grooming behaviour (mean ± standard deviation), referring to itch on each side of the body

	1st observation	*2nd observation*
Right		
Negative control	0.11 ± 0.10	0.08 ± 0.06
D. pruriens 30 CH	0.15 ± 0.17	0.13 ± 0.11
Positive control	0.17 ± 0.13	0.15 ± 0.15
D. pruriens 6 CH	0.21 ± 0.16	0.11 ± 0.12
Left		
Negative control	0.10 ± 0.09	0.08 ± 0.05
D. pruriens 30 CH	0.14 ± 0.16	0.13 ± 0.10
Positive control	0.16 ± 0.12	0.14 ± 0.14
D. pruriens 6 CH	0.20 ± 0.15	0.11 ± 0.12

Each animal was observed for 5 min. ANOVA.

Table 9 Delta value of behaviour scores between the 15 and 30 days of treatment (value measured on the 30th day, subtracted from the value measured on the 15th day)

	Immobilization	*Locomotion*
Negative control	0.17 ± 0.39	−17.3 ± 17.55
D. pruriens 30 CH	0.52 ± 0.37	−17.0 ± 22.14
Positive control	0.23 ± 0.94	−17.3 ± 15.34
D. pruriens 6 CH	0.02 ± 0.20	−6.6 ± 29.89

This index was calculated to detect the possibility of temporal variations in the appearance of any pathogenetic symptoms (mean ± standard deviation). ANOVA.

Table 8 Water (ml) and food (g) consumption in proving model

	Negative control	*Positive control*	*D. pruriens 30 CH*	*D. pruriens 6 CH*
Food consumption	116.37 ± 14.88	115.89 ± 15.50	111.88 ± 12.58	122.45 ± 13.80*
Water consumption	136.83 ± 23.60	132.56 ± 20.98	126.18 ± 18.62	147.11 ± 30.69**

Mean ± standard deviation of daily consumption.
*ANOVA, Tukey–Kramer, $P < 0.05$ relative to the *D. pruriens* 30 CH.
**ANOVA, Tukey–Kramer; $P < 0.01$ relative to the *D. pruriens* 30 CH.

Table 10 Frequency of idiosyncratic animals, showing behavioural scores more than two standard deviations from the group mean

	Locomotion	*Frequency of Grooming*	*Time of Grooming (min)*	*Rearing*	*Fecal Boli*	*Freezing*	*Grooming*	
							Right	*Left*
Negative control ($n = 10$)	0	0	0	0	0	1	0	0
Positive control ($n = 15$)	0	1	0	1	0	3	0	0
D. pruriens 30 cH ($n = 15$)	1	0	1	1	1	0	2	2
D. pruriens 6 cH ($n = 15$)	0	2	2	2	2	1	2	1

the average duration of trials was about 34 days. But, it is not known if this 30-day period of treatment would be long enough for symptoms to arise in rats. In human beings, pathogenetic trials with a large range of duration have been reported.[53–55] In animal experiments, study periods of 4–12 days can be found, as in the study of *Oenanthe crocata*;[56] 35 days for the *Kreosotum* study;[12] 45 days for *Propolis*;[28] and 60 days in the pathogenetic trial of *Arsenicum album*.[27] In this experiment, *D. pruriens* was given to rats for 30 days, a longer period should be considered for further studies.

Another difficulty of behavioural study methods arises from the influence of environmental factors on the analysis of biological parameters, as Fernandes[57] discovered in his experiment, in which the simple transfer of rats to another room produced measurable alterations in several biological parameters. Majerowicz[41] and Andersen *et al*[23] also obtained similar results. To help avoid this kind of bias in the present study, only two people were allowed to handle the animals during the experimental period.

Although the results did not show pathogenetic effects, it is important that the search go on, and methodological innovations allowing the analysis of multiple factors are developed.

Conclusion

This model demonstrates therapeutic effects of *D. pruriens* in homeopathic preparations, but not pathogenetic effects.

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