

# The effectiveness of benzyl benzoate and different chemicals as acaricides

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Several observations have indicated that house-dust mites (HDM) play an important role in allergic diseases. Thus, the primary form of treatment should aim at reducing exposure to HDM for these patients. Allergen-avoidance measures in homes have been demonstrated to be beneficial in decreasing the risk of sensitization, severity of symptoms, bronchial reactivity, and basophil sensitivity. Various chemical methods, as well as physical measures, have been tried to eliminate mite allergens from house dust. However, none have gained wide acceptance because of the lack of effectiveness and safety, and the high cost. It is clear that new approaches are required for effective long-term control of HDM allergens. This study compared the acaricidal activities of phenyl salicylate, tea leaf extract (high tannic acid content), and essential oils (eucalyptus and laurel) with that of benzyl benzoate. The contact, short-duration persistence, and residual effects of various concentrations of these chemicals and benzyl benzoate were assessed in laboratory conditions with specially designed wells. Our data suggest that benzyl benzoate may not be effective when applied according to the manufacturer's instructions, but may be effective when applied more frequently (i.e., three to four times a year) and for longer periods (up to 24 h) even with lower concentrations (0.4%). Essential oils were shown to have little acaricidal activity, but virtually no effect was observed with tea. Among the chemicals used, phenyl salicylate seems to be promising as an alternative acaricide.

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Allergy is a serious medical problem worldwide. During the last 30 years, there has been increasing evidence that indoor allergens play an important role in the etiology of asthma and perennial rhinitis, as well as atopic dermatitis (1). Since 1964, an increasing number of observations have identified mites of the genus *Dermatophagoides* as a major pathogenic or, more precisely, allergenic source in house dust (2).

In genetically susceptible individuals, exposure to allergens and particularly to house-dust mite (HDM) allergens has been quantitatively associated with increased basophil sensitivity, risk of sensitization, and asthma severity (3). These results have suggested that reducing such exposure should be a primary form of treatment for these patients.

Several trials of allergen-avoidance measures in houses have demonstrated significant clinical benefit: decrease in asthma symptoms and bronchial reactivity (4, 5). These measures include encasing bedding in hypoallergenic covers, removing carpets, replacing upholstered furniture, washing clothing

and bedding in hot water, ventilation, wet and dry vacuum cleaning, and air filters. Controlling indoor temperature and humidity is also important because mites are highly susceptible to water loss, an effect that increases at higher temperatures (6, 7).

In addition, many different chemicals including acaricides, liquid nitrogen, tannic acid, and essential oils have been applied to eliminate allergens from carpets, upholstery, and bedding (8–12). Benzyl benzoate is a commercially available acaricide (13). More recently, acaricides incorporating tannic acid to denature fecal allergens have been demonstrated to be more effective, decreasing the allergenicity by 1000-fold (14, 15).

Although new approaches to allergen control are clearly welcome, none have gained wide acceptance. Compounds such as phenyl salicylate of similar chemical structure to benzyl benzoate could be of great use in mite control. Leaf extract of tea, *Thea sinensis* L. (*Camellia thea* Link), which contains 10–24% of tannin, had never been considered as a denaturant and acaricide.

The present study was designed to investigate the acaricidal effects of these chemicals and compare their effectiveness at different concentrations on HDM. In this paper, we report the results of three studies undertaken to determine the roles of benzyl benzoate, phenyl salicylate, *T. sinensis*, and essential oils (laurel, eucalyptus) as acaricides over a 3-month period.

## Material and methods

### Study design

The chemical compounds tested as acaricides in this study were benzyl benzoate (Fluka, Switzerland), phenyl salicylate (Fluka, Switzerland), eucalyptus oil and laurel oil (Sigma Chemicals, St Louis, MO, USA), and tea as a source of tannic acid. Acetone (Merck, 2500 Reinst) was used as a solvent because of its volatile character. The dry tea leaf sample used in this study was found to contain 9.5% tannin by spectrophotometric analysis with leather powder (16). The concentrations of *T. sinensis* were determined by diluting the stock solution with the appropriate amount of acetone. The acaricidal activities of benzyl benzoate (concentrations of 3.2, 0.8, 0.4, 0.2, 0.1, 0.05%), phenyl salicylate (0.8, 0.4%), essential oils (3.2, 0.8%), *T. sinensis* (9.5, 3.8, 1.9%), and acetone-only solution (control) were evaluated by comparative counts, at regular intervals, with *Dermatophagoides farinae* populations.

Mites used in this study were obtained from laboratory cultures of beard shavings and fish meal at 25°C, 75% relative humidity (RH), in the dark. In experiments to test acaricidal activity, groups of 20–30 live mites were added to specially designed wells using a fine paintbrush with the aid of a low-power inverted microscope. These wells consisted of approximately 1 cm<sup>3</sup> space covered on one side with black filter paper and on the other side with a glass slide (Fig. 1) (17). Before the mites were added, the wells and the slides were immersed in different test solutions to form a film, as determined by each step of the experiment. Harvesting from active cultures to each well was performed in triplicate; in all cases, fish meal particles were then added as food.

The following were investigated in laboratory conditions:

- 1) contact effect
- 2) short-duration persistence of acaricidal activity
- 3) residual effectiveness of the chemicals.

All cultures were placed at room temperature (25°C), 75% RH. For evaluation of contact acaricidal activity, the mites were classified as dead or alive on the basis of mobility after 15 min; 1, 3, 24, 48, and 72 h; and 1 week under a binocular light microscope.

For determination of the short-duration persistence of the acaricidal activity, another set of wells and lancets was prepared for each dilution of chemicals – excluding essential oils – to form a film, as described above. Mites were then added to the wells at 24, 48, and 72 h (3 consecutive days) after coating. Counting was done at 1, 2, and 24 h and week 1 of each test day.

Another experiment using benzyl benzoate (concentrations of 3.2, 0.8, 0.4%), phenyl salicylate 0.8%, and laurel oil 3.2% was performed to assess the residual effectiveness up to 3 months. In this test, higher benzyl benzoate concentrations were selected, on the assumption that sufficient knowledge about its residual effect could be gained. The wells and the lancets were layered with these chemicals and kept in laboratory conditions until the mites were introduced at monthly intervals during the next 3 months. After the mites were added, these wells were returned to optimal conditions at 25°C, 75% RH, and in darkness. “Live” (white, mobile) mites were counted 1 week after treatment. Only mites that showed no sign of activity, even on prodding with a blunt needle, were categorized as “dead” (brown, shrunken).

### Statistics

A hypothesis test for two proportions from independent groups (*z*-test) and chi-square analysis were used to examine the homogeneity of the control data and the differences in the number of mites killed by each dilution of chemicals over different time periods. A *P* value of <0.05 was regarded as significant.

## Results

### Contact effect of the acaricides

Benzyl benzoate killed all mites within 15 min with the 0.8% dilution, 1 h with the 0.4% solution, and 3 h with the 0.1% solution, showing almost equally quick acaricidal activity for all the concentrations used (Table 1). Phenyl salicylate killed most of the mites in 1 h, and total acaricidal activity (100% mortality) was seen after 3 h, not significantly differing from low concentrations of benzyl benzoate (i.e., 0.1%) (*P* > 0.05). Although the effectiveness of essential oils increased with longer exposure times, most mites were killed in 24 h by 3.2% laurel oil. However, the acaricidal activity of 3.2% laurel oil was as effective as 0.05% benzyl benzoate after 1 week of exposure. This type of acaricidal activity was not observed with eucalyptus. Among the other compounds used, 9.5% tea caused slow acaricidal effect at the end of the first day; with 3.8% tea, this was seen after 3 days, significantly differing from

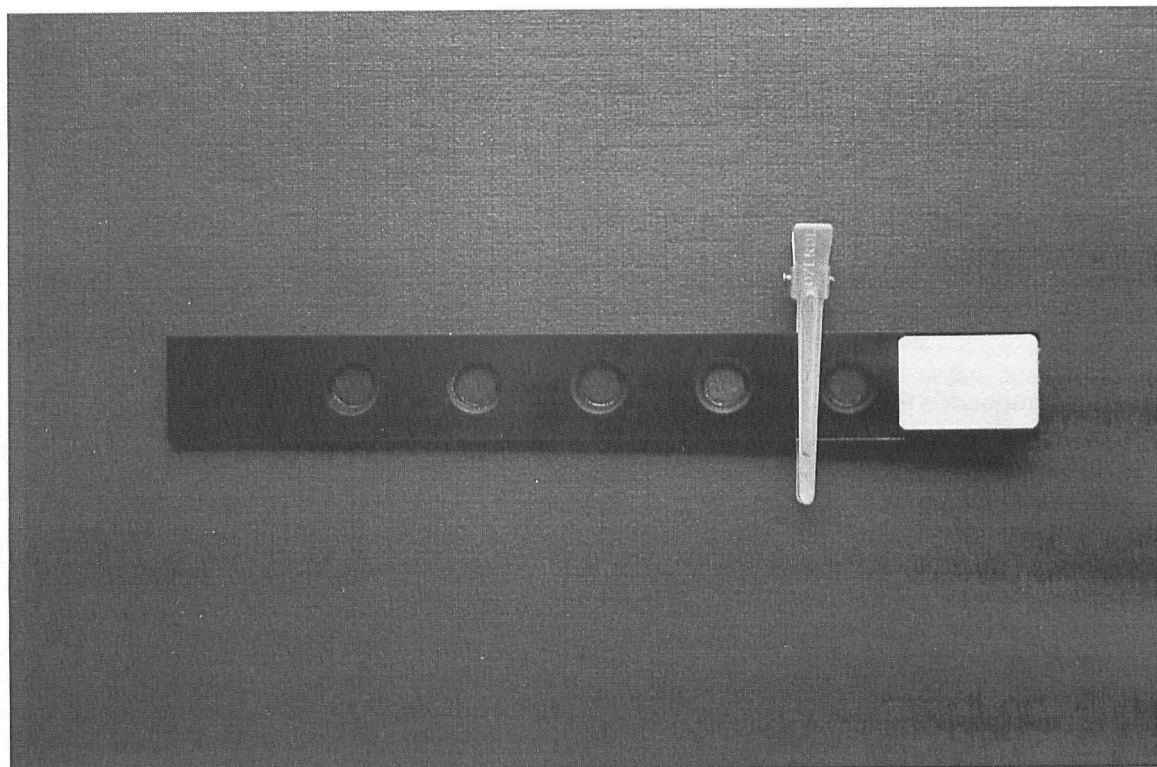


Fig. 1. Specially designed wells used in study.

Table 1. Percentage of mites killed by chemicals in different time periods

| Chemical       | Dilution (%) | Percentage of mites killed by chemical |       |      |       |       |       |        |
|----------------|--------------|----------------------------------------|-------|------|-------|-------|-------|--------|
|                |              | 15 min                                 | 1 h   | 3 h  | 24 h  | 48 h  | 72 h  | 1 week |
| Acetone        |              | 0                                      | 0     | 5.45 | 10.91 | 10.91 | 12.73 | 14.55  |
| Laurel oil     | 3.2          |                                        |       |      | 77.50 |       |       |        |
| Laurel oil     | 0.8          |                                        |       |      | 12.70 |       |       |        |
| Eucalyptus oil | 3.2          |                                        |       |      | 2.38  |       |       |        |
| Eucalyptus oil | 0.8          |                                        |       |      | 2.90  |       |       |        |
| BB             | 0.8          | 100                                    |       |      |       |       |       |        |
| BB             | 0.4          | 67.65                                  | 100   |      |       |       |       |        |
| BB             | 0.1          | 71.43                                  | 92.86 | 100  |       |       |       |        |
| BB             | 0.05         |                                        |       |      | 63.64 | 92.73 | 96.36 | 98.18  |
| PS             | 0.8          | 2.47                                   | 92.59 | 100  |       |       |       |        |
| PS             | 0.4          | 5                                      | 91.25 | 100  |       |       |       |        |
| Tea (TA)       | 9.5          | 0                                      | 13.11 |      | 18.03 | 36.07 | 40.98 | 62.30  |
| Tea (TA)       | 3.8          | 1.35                                   | 5.33  |      | 8     | 14    | 32    | 29.33  |
| Tea (TA)       | 1.9          | 2.86                                   | 7.14  |      | 11.43 | 11.43 | 11.43 | 27.14  |

BB – benzyl benzoate; PS – phenyl salicylate; TA – tannic acid.

the control ( $P < 0.05$ ). However, there was no significant change in the number of live mites after exposure with any concentration of tea.

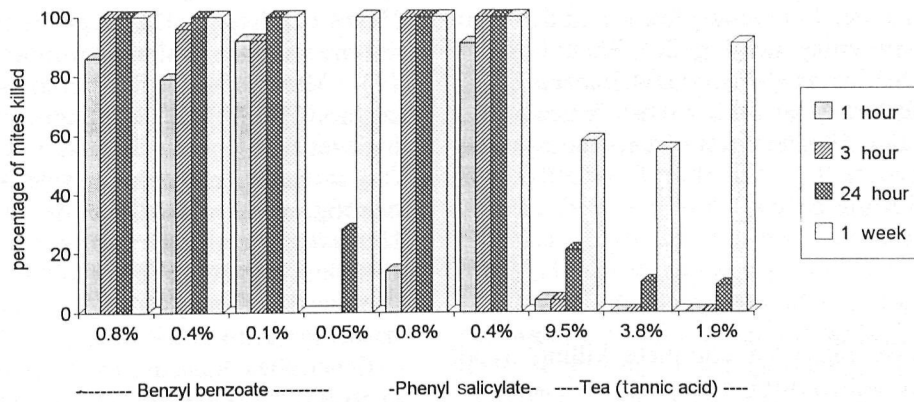
#### *Short-duration persistence of the acaricidal activity*

*Twenty-four hours.* Although most mites were killed after 1 h of exposure by different benzyl benzoate dilutions showing similar acaricidal activity, the effectiveness increased with higher concentrations

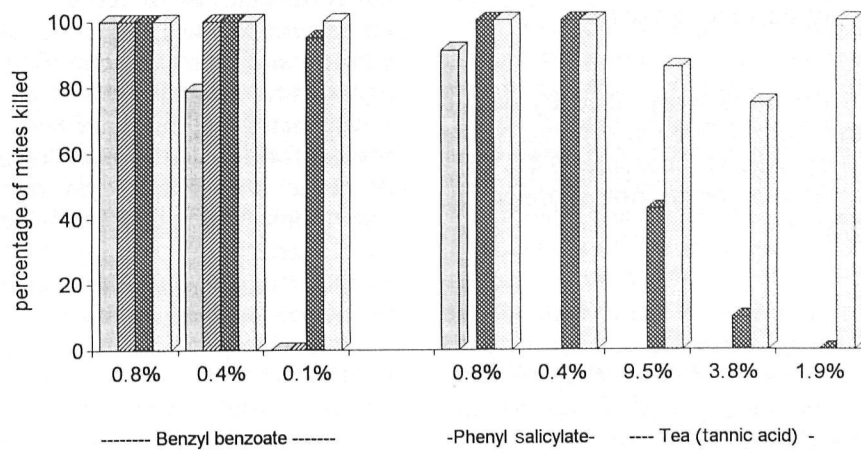
as well as longer exposure ( $P < 0.01$ ) (Fig. 2a). Phenyl salicylate killed all mites after 3 h, as did 0.8% benzyl benzoate. No acaricidal activity was seen with tea dilutions of 1.9% and 3.8% within a short exposure period. Although the effectiveness of tea at any concentration slightly increased after 1 week, this was significantly less than the other chemicals ( $P < 0.01$ ).

*Forty-eight hours.* The high concentration of benzyl

a.



b.



c.

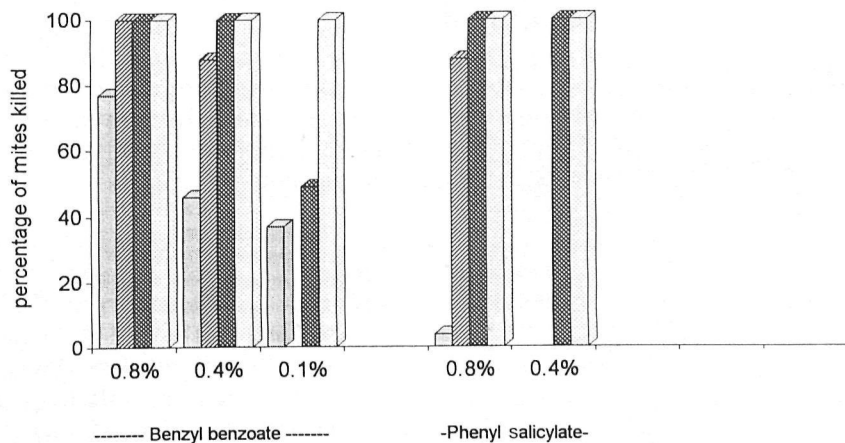


Fig. 2. Short-duration persistence of acaricidal activity (% mites killed) was examined at intervals of a) 24 h, b) 48 h, c) 72 h.

benzoate (0.8%) killed all mites, and most were killed by the concentration of 0.4% within 1 h of exposure, even after 48 h of coating (Fig. 2b). However, the acaricidal activity observed with the low concentration of benzyl benzoate (0.1%) was quite slow ( $P < 0.01$ ). All mites were killed by phenyl

salicylate within 24 h, that is, more effectively than 0.1% benzyl benzoate ( $P < 0.01$ ). The acaricidal activity of all the tea dilutions was observed with longer exposure times as was seen in the first 24 h. Thus, no significant change in the numbers of live mites was observed.

*Seventy-two hours.* The total acaricidal activity was seen after only 3 h with the highest concentration of benzyl benzoate, but mainly by the end of day 1, as with phenyl salicylate (Fig. 2c). Phenyl salicylate was as effective as 0.4% benzyl benzoate, but much less effective than with earlier exposures at this time period. The persistent acaricidal activity of benzyl benzoate 0.1% fell sharply (<50%) until day 1 after 72 h of coating.

### Residual activity

*First month.* We observed complete killing in all cases – except with laurel oil – within 1 week of exposure. A few dead mites were collected from the wells treated with laurel oil, a result not significantly different from that of acetone ( $P > 0.05$ ) (Fig. 3).

*Second month.* All the mites were killed by benzyl benzoate 3.2% and benzyl benzoate 0.8%, while most were killed by benzyl benzoate 0.4% and phenyl salicylate 0.8%, a result not significantly different from month 1.

*Third month.* Most mites were killed with all the concentrations of benzyl benzoate and phenyl salicylate. The effectiveness of phenyl salicylate persisted throughout the study period. However, the residual activity of benzyl benzoate significantly decreased at higher concentrations, leading to a significant difference between months ( $P < 0.01$ ).

## Discussion

HDM can live in all kinds of textiles, and they feed mainly on skin scales from man or animals (3, 7, 11). Mattresses, upholstered furniture, carpets, cushions, clothing, and cuddly toys may contain high levels of mite allergens (9). Their life span is 2–4 months. In autumn, with the start of central heating, most HDM die as a result of dry air. However, the allergic reactions are at their peak at this time because the equally allergenic excrement produced remains, while the brood survives, thereby ensuring continued existence.

Given the conclusive evidence of the role of HDM exposure in sensitization and the development of asthma, there have been intensive efforts to develop better techniques to reduce mite exposure. There are several chemicals that kill HDM in a laboratory culture, and many that are used to control mites in agriculture. Several acaricides, mainly based on benzyl benzoate, that have been widely used to treat scabies for at least 50 years have been marketed for domestic use. Despite varying results, significant reductions in allergen levels have been reported with each preparation. Among the chemicals available in the struggle to reduce the mite population, the acaricides lead to an elimination of 65–100% of mites in culture. However, the efficacy of acaricides is influenced by various factors within each home and by the delivery systems (6, 18). Although these issues have not been addressed adequately, it is logical that these chemicals need to reach live mites in order to be effective in homes.

% mites killed

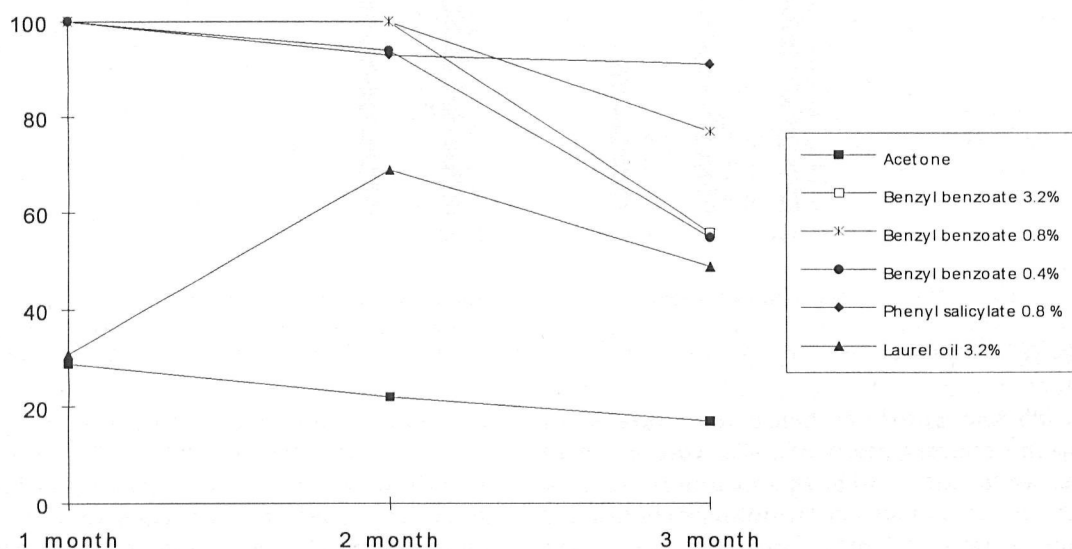


Fig. 3. Residual effect of acaricidal activity.

The results of some clinical trials on benzyl benzoate have been encouraging, especially as its effects were claimed to persist for up to 6 months after one or two treatments per year according to the manufacturer's recommendations (13). However, there were no significant changes in lung function and medication or mean allergen levels in some other studies. Subsequent experiments suggest that higher rates of application are more effective (19). Thus, it is estimated that applications of benzyl benzoate would be required every 3 months to decrease the mite-allergen concentration indoors (18). Our study demonstrates that even low concentrations of benzyl benzoate kill mites on contact. When benzyl benzoate 3.2% was used more often than the manufacturer's recommended rate of application, a high reduction in live mites, relative to the baseline, was observed. This was also confirmed with the minimum effective dose used in this study. It is important to note that the duration of the acaricidal activity of benzyl benzoate concentrations much lower than what is found in commercially available products (0.8% and 3–3.5%, respectively) has been shown to persist for up to 3 months.

Another important matter that must be kept in mind is that research on new alternatives should focus on the least toxic drugs. After higher exposure, serious concern could arise about the side-effects of such chemicals. Most of the chemicals proposed to kill mites are considered to be safe; therefore, evaluating the toxicity of any chemical product is a complex process and depends on the way it is used. Although no resistance has been reported by pyroglyphid mites, in agricultural use mites have developed resistance to benzyl benzoate. Phenyl salicylate can be assumed to be a promising alternative. However, the use of phenyl salicylate as a clinical acaricide necessitates rigorous, longitudinal, randomized safety/toxicity studies on continuous long-term exposure.

Tannic acid has been traditionally used as a health measure in some parts of China. Putting tea leaves on carpets has been recommended for many years (20). The acid is marketed in 1% and 3% solutions. It very rapidly reduces the allergenicity of dust by its protein denaturing activity (21, 12). Therefore, the effect can be only temporary (i.e., weeks).

Turkey is a country rich in tea plants, mainly in the northern part where HDM are an important cause of allergy because of the moist climate. For these reasons, it could be beneficial to try tea as an acaricide. With various tea solutions up to 9.5% tannin (much higher than the regular amount found in market products), hardly any acaricidal effect has been observed. Besides, because of its dark stain

and sticky residue, tea would be impracticable as an acaricide no matter how much cheaper it is than other chemical compounds.

Essential oils were not demonstrated to be promising acaricides in this study. Although some oils have been shown to kill pyroglyphid mites by other authors, the mechanism of killing is still not clear (11).

It is disappointing that no universally effective chemical treatment has been developed to control mites. However, this is not only because of the lack of effective and safe acaricides, but also because of the high cost and difficulty in applying these chemicals. The use of an acaricide only kills mites; therefore, it must be followed by a thorough vacuum cleaning of the treated surfaces to remove the mite-allergen pool.

In conclusion, applying lower concentrations of benzyl benzoate three to four times a year will be within the safety range in killing mites effectively. Effective long-term control of HDM allergen levels with phenyl salicylate also requires substantial further studies. However, the application of allergen-avoidance measures to symptomatic asthmatic patients should be encouraged to reduce the use of chemical treatments.

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