# Stimulation of humoral immunity in mice by some commercial fragrances

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***Abstract***

*The in vivo effect of some commercial fragrances on humoral immunity in mice was studied. Stimulatory effect of inhalation on anti-SRBC antibody production was presented by eau de toilette Lily of the Valley, and eau de toilette WARS. No effect of eau de toilette CREATION was observed.*

***Key words:*** *fragrances, humoral immunity, mice.*

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## Introduction

Natural odors and synthetic fragrances may influence animals' and humans' everyday activity and health. In our previous papers we presented the effects of some natural essential oils and synthetic volatile substances (undecanones) on specific and non-specific immunity in rodents [1-11]. As it has been shown by us recently, the majority of these substances, introduced to mice by inhalation has increased their cellular and humoral (antibody response to antigen sheep red blood cells, SRBC) immunity. The mechanism of this effect is not clear, but there are reports that a smell is information for the immune and nervous system to change their level of activity. Immune cells receive signals via receptors for neurotransmitters and some hormones on their surface. This transmission can be performed by two ways: through the central nervous system (CNS) and by the hypothalamic – pituary – adrenal (HPA) pathway [2-20]. Commonly used perfumes, deodorants and various cleaning and cosmetic products contain a lot of fragrant substances. The aim of the present study was to examine the effect of three fragrances on antibody response of SRBC-immunized and inhaled mice.

## Materials and Methods

### Fragrances

Eau de toilette Lily of the Valley, eau de toilette WARS, eau de toilette CREATION.

**Mice.** The study was performed on 10-12 weeks old female Balb/c mice, weighing 25-28 g, delivered from the Polish Academy of Sciences.

**Study of antibody production.** Mice were immunized with 10% SRBC suspension (0.1 ml intraperitoneally), and subjected to inhalations for 3 consecutive days, according to the following scheme: 5 mice in one cage, 5 drops of tested compound for 60 minutes, the cage covered by linen during inhalation. The cages with control mice were accordingly covered by linen for 60 minutes.

Mice were bled in anaesthesia (3.6% chloral hydrate), from retroorbital plexus, 7 days after immunization. The antibody level was evaluated with haemagglutination assay in inactivated (56°C, 30 min) sera. After performing a series of sera dilutions, 0.5 % SRBC were added and the mixture was incubated for 60 min at room temperature, then centrifuged (10', 150 g) and shaken. The hemagglutination titer was evaluated in a light microscope – as the last dilution in which at least 3 cell conglomerates were present in at least 3 consecutive fields at objective magnification 20x [21] and the results were presented as log titers. Statistical analysis was performed by *t*-Student test (Statistica 8.PL) and verified by one-way ANOVA (GraphPadInStat3). Experiments were approved by the Local Ethical Committee.

## Results

The effect of fragrances on antibody production is presented on the Table 1 and on the Figure 1. According to

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**Table 1.** Statistical differences between the control and experimental groups

| Mean | SD | N | SEM | Ref. value | t | DF | p |
|---|---|---|---|---|---|---|---|
| 4.800000 | 1.032796 | 10 | 0.326599 | 4.800000 | 0.000000 | 9 | 1.000000 Control |
| 5.900000 | 0.737865 | 10 | 0.233333 | 4.800000 | 4.714286 | 9 | 0.001098 *** L |
| 5.700000 | 0.823273 | 10 | 0.260342 | 4.800000 | 3.456996 | 9 | 0.007195 ** W |
| 5.200000 | 0.788811 | 10 | 0.249444 | 4.800000 | 1.603567 | 9 | 0.143273 n.s. CR |

*L – Lily of the valley; W – WARS; CR – Creation.*

ANOVA the P value is 0.0227, considered significant (variation among column means is significantly greater than expected by chance). Except eau de toilette "Creation", inhalation of mice with other tested substances significantly stimulated this parameter of humoral immunity.

## Discussion

The results of the present study confirm our earlier findings, that volatile substances introduced to the animals by inhalation may change their immunological response to the antigens [1-11]. In this paper we present for the first time the evidence of immunostimulatory activity of some popular fragrances- eau de toilette for women "Lily of the Valley" (L) and eau de toilette for men- "WARS "(W). The fact that not all fragrances increased humoral immunity in inhaled mice suggests that it is not a direct stressing effect of smelling stimulus. Similarily, we have observed previously that fragrant substances would be stimulatory, inhibitory or neutral in respect to the immunological response of mice against foreign antigens. For example, some essential oils or their compounds stimulated cellular and humoral immunity, some were indifferent, and some exerted inhibitory effects [1-5, 9, 11].

In conclusion, we suppose, that immunostimulatory fragrances may be beneficial for additional treatment of patients suffering from various infections. They might be also desirable for patients with immune disturbances, for example in cancer patients after treatment with cytostatics, when the immune system is depressed. So, our present and previous studies reveal that the proper choice of natural or synthetic compound as a fragrance or food additive may be important.

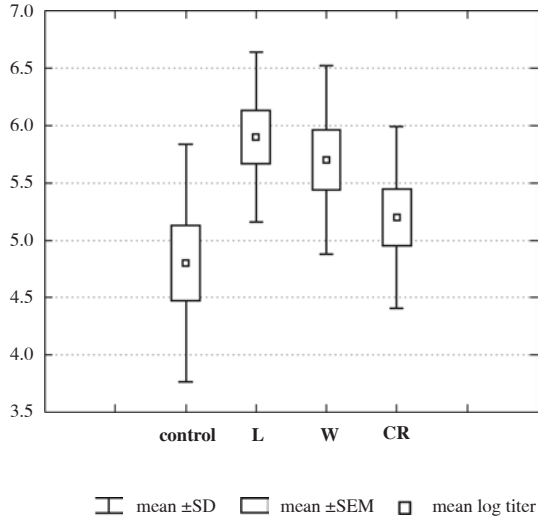


**Fig. 1.** The effect of inhaled fragrances on antibody response in mice. L – Lily of the valley; W – WARS; CR – Creation.

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