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# Homeopathic medicine Cantharis modulates uropathogenic *E. coli* (UPEC)-induced cystitis in susceptible mice

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ABSTRACT

*Objective:* This is a random blinded placebo controlled murine experimental model to study the effects of Cantharis 6 CH, a homeopathic medicine, on *E coli*-induced cystitis.
*Methods:* 24 adult susceptible female BALB/c mice were inoculated with *E coli* – UPEC O4:K-:H5 by a transurethral catheter. Cantharis 6cH or vehicle (placebo) was offered to mice by free access into the drinking water (1:100), during 24 h after infection. Spleen, bladder and kidneys were processed for quantitative histopathology after immunohistochemistry, using anti-CD3, CD79, MIF, NK and VEGF antibodies; the cytokines present in the bladder washing fluid were measured using a LUMINEX-Magpix KIT. Mann-Whitney and Fisher exact test were used as statistical analysis.
*Results:* Cantharis 6 CH increased IL12p40, IFN-γ and decreased IL10 concentrations in the bladder fluid ($p \leqslant 0.05$); in the bladder mucosa, it increased the ratio between B and T lymphocytes (31%) and between B lymphocytes and MIF+ macrophages (57%, $p \leqslant 0.05$). In the pelvis, instead, it decreased the B/T cells ratio (41%, $p \leqslant 0.05$) and increased the M1/M2 macrophage ratio (42%, $p \leqslant 0.05$). No differences were seen in the kidney and spleen analysis.
*Conclusion:* The inverted balance of inflammatory cells and cytokines in bladder and pelvis mucosa shows specific local immune modulation induced by Cantharis 6cH.


## 1. Introduction

*Escherichia coli* is one of the agents commonly involved in digestive and extra-intestinal infections, such as those in the urinary tract infection (UTI), being this pathotype called UPEC (uropathogenic of *E. coli*) [1]. The infection happens when uropathogens ascend and colonize the lower or upper parts of the urinary tract, originating cystitis and/or pyelonephritis [2]. That is due to the unbalance of the host-parasite relation which involves two simultaneous factors: the rupture of the hosts' organism's defense mechanisms and the presence of sufficient number of virulent microorganisms capable of adhering, multiplying and persisting in a portion of the urinary tract [3]. Since uropathogens present great resistance to available antibiotics, exploring alternative strategies for managing UTI is a theme of interest [4–7].

Two adhesins participate in uropathogenic processes: type 1 fringes and pili, both involved in bacteria colonization in the urinary tract [8–10]. The interaction between *E. coli* and the hosts' tissue, however, also depends on the immune condition. It is known that B-lymphocytes produce immunoglobulins capable of fixing complement and inducing bacterial lysis; the pro-inflammatory macrophages (M1) are capable of secreting cytokines, that amplify the interaction between lymphocytes and phagocytes, enhancing the presentation of antigens and specificity of the immune response [11]. A recent study developed *in vitro* showed that the interaction between B-lymphocytes and macrophages can ease the expression of pro-inflammatory cytokines by the later [12].

The development of medications capable of optimizing those interactions and, thus, decreasing the vulnerability of hosts bearers of UPECs to ascendant and systemic infection, would be very valuable for controlling UTI in susceptible populations. Homeopathy is

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appointed as a potentially useful tool in such cases. Recent results show that different homeopathic medications are capable of modulating the interaction macrophage-parasite *in vivo* and *in vitro*, modifying the dynamics of cellular migration between lymphoid organs and the infection site, as well as the phagocytic activity [13–17]. This dynamic was also reported to Cantharis in an experimental UTI [18].

Other studies have demonstrated the action of homeopathic medicines in inflammatory cells. In [19], Falkowski-Temporini and co-workers showed the increase in megakaryocytes and Kupffer cells, as well the predominance of Th1 response (increased TNF-a, IL-10, TNF-a/IL-4, TNF-a/IL-17, and decreased IL-6, IL-6/IL-4) were seen in mice infected with *Trypanosoma cruzi* and treated with *Lycopodium clavatum*. Besides the reported studies performed by our group [20,21], other studies about the properties of homeopathic products on the regulation of inflammatory process has been recently published [17,22–26].

The choice of the homeopathic medicine *Cantharis vesicatoria* occurred because of its characteristic action on the urinary tract, mainly in situations where the constant urging to urinate is seen, as well the presence of burning pain and frequent painful urination [27]. Despite of the fact that the traditional use of *Cantharis vesicatoria* is very stablished in homeopathic medicine, few scientific studies about its mechanisms are seen in the literature. This is the main contribution of this work, show some aspects of the mechanisms involved in its therapeutic effect, regarding to the regulation of local inflammatory process. The 6 CH dilution (or potency) was chosen because this is routinely used in the clinical practice, in acute cases, as reported by Fontes et al. [18] and Tarevnic e Pinto [28].

The aim of this study was to verify, in a murine experimental model, if homeopathic medicine Cantharis can interfere in the physiopathological aspects of *E. coil*- induced cystitis.

## 2. Methods

### 2.1. Animals and ethics

The animals used in this study were in accordance with Brazilian standards and ethic procedures regarding the use of laboratory animals. The project was approved by Paulista University ethics committee (CEUA-UNIP) under protocol nº 062/11 CEP/ICS/UNIP, in February 9th, 2012. The study used Balb/c female adult mice (N = 32) kept in controlled conditions of temperature (22–26 °C) and humidity (50–65%), in micro-isolators (Techniplast®) located in the SPF (Specific Pathogen Free) vivarium of UNIP's Research Center. The animals were fed *ad libitum* with water and food and were kept with light/dark periods of 12/12 h (light period at 7:00 AM). The use of animals in this study was inexorable, since the balance of cell migration among different levels of urinary tract has to be observed in a systemic approach.

### 2.2. Murine model of ascendant urinary infection

This protocol was defined after a series of previous pilot studies. The *Escherichia coli* strain JJ079, prototype of urosepsis (UPEC O4: K-:H5, genotype *pap*+, *sfa*+, *fimH*+, *hly*+, *cnf*1+, *fyuA*+, *traT*+, *malX* +), was cultivated overnight in LB medium (DIFCO®), centrifuged for 30 min at 15,000 RPM, 25 °C and suspended in sterile PBS to obtain the concentration of $7.5 \times 10^{11}$ UFC/mL (5,0 Mac Farland scale). The pathogenicity of bacteria was checked before all the experimental procedures, by the hemagglutination test. The bacterial suspension was inoculated in each animal through a transurethral sterile catheter under sterile conditions. The mice were deprived of water for 4 h to warrant the emptiness of the bladder before inoculation. Two hours before, all animals were treated intraperitoneally with 5 mg/kg of disodium phosphate dexamethasone (0.1 ml/10g of body weight) in order to increase their susceptibility to bacterial infection. For the bacterial inoculation, the sedation was performed with association of two parts of xylazine 2% and one part of ketamine chlorhydrate 10%. This mix was diluted once again in 4 parts of sterile physiologic solution, to be administered intraperitoneally (0.4 ml/10g body weight). After verifying sedation, the urethral inoculation was carried out with 50 μL of UPEC JJ079 suspension ($7.5 \times 10^{11}$ UFC/mL), kindly inserted into the bladder with a 22G probe. After 24 h, the animals Were euthanized with an association between xilazine (50 mg/kg) and ketamine (125 mg/kg), injected intraperitoneally.

### 2.3. Preparation of Cantharis 6CH and 20% hydro-alcoholic solution (vehicle)

The homeopathic medication Cantharis 6cH is obtained from a beetle called *Cantharis vesicatoria*, and the mother tincture is obtained from the maceration of such insect. Herein, the medication preparation was prepared in an ANVISA (Brazilian Agency of Health) accredited commercial pharmacy and the method used to prepare this medication followed the Brazilian Homeopathic Pharmacopeia, 3rd edition, 2011 [29]. Thus, serial 1:100 dilutions of the matrix (*Cantharis versicatoria*) was made in 20% alcohol followed by automatic 100 vertical automatic mechanical agitations in a proper device (Autic®). The samples of the medications are stored in sterile amber flasks and kept in room temperature. This procedure was repeated 6 times in order to reach the final flask (6CH or 6th centesimal dilution following Hahnemann's method) to be used in the experimentation. For the preparation of the control, 20% hydro-alcoholic solution, the same dilution procedures were performed using only alcohol (vehicle). After the dilution of 1: 100 into the drinking water, there was no further agitation and the liquid just remained available to the animals.

### 2.4. Experimental design

The experimental animals were divided in 2 groups, with N = 12 per group, which were:

- ***Placebo group***: treated with the vehicle;
- ***Experimental group:*** treated with Cantharis 6 CH.

An additional ***Control Group***, N = 8, composed by no inoculated nor treated animals, was also added for standardization of the histological features of the colony. All treatments started immediately after bacteria inoculation up to 24 h. Medicines were added 1:100 into the drinking water and offered to mice as free access. The pH of each drinking bottle was measured and no alteration was observed after the drugs adding (pH = 7.5 to 7.7). The free access in the water is a kind of homeopathic administration strategy used experimentally in previous studies and it is particularly useful in population veterinary medicine, as shown in *E coli* infection control in pig farms [13,14,16,30].

### 2.5. Necropsy and material harvesting

A unique blood sample was harvested with calibrated loop and seeded directly in a MacConkey agar (DIFCO®) plate, for UFCs counting. The bladder was divided in two equal parts. The first part was fixed in 8% paraformaldehyde for posterior histological procedures. The second fragment was grinded in a drop of sterile PBS and the washing fluid was frozen for posterior cytokines dosage. The histology was performed according to conventional paraffin-embedded inclusion and hematoxylin-eosin (HE) staining method.