



Short communication

Mast cells respond to urticating extract from lepidoptera larva *Morpheis ehrenbergii* in the rat



María Fernanda Galicia-Curiel ^a, J. Luis Quintanar ^b, Mariela Jiménez ^a,
Eva Salinas ^{a,*}

^a Laboratory of Immunology, Department of Microbiology, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Av. Universidad 940, C.P. 20131 Aguascalientes, Mexico

^b Laboratory of Neurophysiology, Department of Physiology and Pharmacology, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Av. Universidad 940, C.P. 20131 Aguascalientes, Mexico

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ABSTRACT

Mast cells and histamine participate in toxic effects of hairs from some caterpillars. This study reports that a crude extract of *Morpheis ehrenbergii* caterpillar hairs induces *in vitro* mast cells activation, triggers the release of histamine and causes a rapid urticarial reaction in the rat skin. Heating of the extract abolishes the inflammatory reaction. These results suggest that the use of antihistamines may improve the adverse skin reactions caused by the Mexican caterpillar *M. ehrenbergii*.

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Mast cells (MCs) are versatile cells of the immune system that contribute to the innate and adaptive defense against pathogens and are involved in allergic responses. MCs are dispersed in many tissues, mainly in that commonly exposed to the external environment, such as skin, airways and gastrointestinal tract. MCs are able to initiate and enhance early responses to environmental threats, including toxins or venom insects (Galli and Tsai, 2010; Rao and Brown, 2008). Activated MCs release different mediators, such as histamine, that are essential for the induction of local inflammatory responses in many tissues (Prussin and Metcalfe, 2006; Salinas, 2012; Salinas et al., 2009).

Lepidopterism or erucism, derived from contact with adult lepidoptera (moths and butterflies) or caterpillars (larval phase of lepidoptera) and/or their products, can result in adverse reactions that vary in severity from mild irritation to severe reactions including anaphylactic shock and death (Hossler, 2010a, 2010b). Some caterpillars are full of microscopic urticating hairs able to penetrate the epidermis and mucous membranes and serve to protect the caterpillar against predators but are also dangerous for animals and men (Novak et al., 1987). The most frequent clinical manifestations they cause are cutaneous, mainly contact dermatitis or urticaria. Explanation for these reactions includes irritant or toxic mechanisms after substance release and the trigger of an allergic immunoglobulin (Ig) E-mediated reaction to different caterpillar proteins (Vega et al., 2011).

Morpheis ehrenbergii is a Mexican butterfly. It is distributed throughout the Pacific coast and in Central Mexico. In its larval stage, the caterpillar is equipped with black irritating hairs able to penetrate the dermis. These

* Corresponding author. Department of Microbiology, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Av. Universidad 940, C.P. 20131 Aguascalientes, Ags., Mexico. Tel.: +52 449 9 10 84 24; fax: +52 449 9 10 84 23.

E-mail addresses: emsalin@correo.uaa.mx, emsalin@live.com (E. Salinas).

hairs protect caterpillars against invertebrate and vertebrate enemies (Vázquez, 1934). Unpublished observation indicates that direct contact with *M. ehrenbergii* caterpillar causes severe skin reactions. In this study we analyzed in the rat the effects of crude extract from hairs of lepidoptera larva *M. ehrenbergii* on cutaneous reaction and *in vitro* on MCs activation and histamine secretion.

For all the experiments *M. ehrenbergii* caterpillars were collected in the wild field of Aguascalientes, México. The hairs of 20 caterpillars were crushed in saline solution and crude extract was obtained by centrifugation. Protein concentration was calculated by Bradford method to use 0.22 µg/µl of protein in all assays and extract was stored at –80 °C. Wistar rats (200–250 g) were housed under controlled environmental conditions and maintained with food and tap water *ad libitum*. All experiments were carried out with adherence to ethical guidelines approved by the Institutional Normative Welfare Standards.

For *in vitro* assays rat MCs were obtained by lavage of peritoneal cavity using HEPES-buffered Tyrode solution supplemented with 1% of BSA and purified using a 30–80% discontinuous Percoll gradient. To evaluate MCs activation, cells (3×10^5) were incubated with crude extract and 0.005% (v/v) ruthenium red solution in sterile PBS at 37 °C for 30 min (Lagunoff, 1972). Positive and negative controls of stimulation included MCs incubated with compound 48/80 (C48/80, 5 µg/ml) plus ruthenium red and MCs incubated with ruthenium red dye alone. Cells were analyzed by light microscopy. For histamine release assay MCs (1×10^5) were stimulated with crude extract for 30 min at 37 °C in supplemented RPMI. Cells incubated without crude extract or with C48/80 (5 µg/ml) were used as control of spontaneous or stimulated histamine secretion. Histamine in supernatant and cell pellets was determined using the o-phthal-dialdehyde reaction and was expressed as percentage of the total cellular histamine content (Shore et al., 1959).

For intradermal (i.d) test, reactions were elicited in the shaved dorsal skin of rats. Animals (three per assay) were i.d injected in three different areas with 50 µl of crude extract or with 50 µl of native and denatured (heated 10 min at 100 °C) crude extracts. A similar volume of saline solution (negative control), histamine (H, 0.004 µg/µl) and C48/80 (0.02 µg/µl) solutions (positive controls) were injected in each rat. Immediately, Evans Blue (34 mg/kg) in 3% saline solution was intravenously injected to all animals. Thirty minutes after crude extract challenge, rats were sacrificed and equal areas from the injected sites (blue spots) were removed and the amount of dye in the tissue samples was colorimetrically determined (Jiménez et al., 2012).

Data were presented as mean ± S.E. Statistical analysis was performed by the Student's *t*-test. Differences were considered statistically significant when $p < 0.05$.

Staining of MCs with ruthenium red allows the evaluation of cell activation, as the dye can only enter cells undergoing exocytosis. At 100× analysis of MCs incubated with ruthenium red activated granules appeared in red color and three MCs states were documented: unstimulated, cells without stained granules and its cell boundary sharply defined; activated, cells with a mixture of non-

stained and red stained cytoplasmic granules and with persisting cell-border; degranulated, cells with all their granules red stained, most extruded from the cell and with loss of their cell boundary (Fig. 1A). The amount of cells in each state was different for spontaneous, crude extract and C48/80 stimulation (Fig. 1B). The percentage of activated MCs in response to crude extract was increased 2.8-fold in relation to spontaneous assay and consequently the number of unstimulated cells decreased 2.4 times versus MCs in the absence of extract (Fig. 1C). The number of degranulated MCs was similar with or without crude extract stimulation, being significantly lower than that obtained by C48/80. In order to verify the extent of exocytosis triggered by crude extract of *M. ehrenbergii*, we measured the release of histamine from MCs. The level of histamine secreted by MCs in presence of crude extract was significantly higher than spontaneous secretion (Fig. 1D). No histamine was detected in extract alone. C48/80 increased 2-fold and 3-fold the percentage of histamine released by MCs in the presence or absence of crude extract. These results indicate that crude extract of *M. ehrenbergii* induces histamine release from MCs, without massive degranulation.

Intradermal skin test is used to determine the magnitude of the inflammatory process generated by an irritating substance. Tissue Evans blue amount indicates extravasation activated by some external agent, in this case a crude extract from *M. ehrenbergii*. Crude extract generated blue inflammatory areas similar in onset (less than one minute) and size to those of histamine and C48/80, although with different color intensity (Fig. 2A). Quantification of extravasated Evans blue revealed that crude extract induced a 2-fold enhancement in the intensity of activated inflammatory process as compared to saline solution (Fig. 2B). Inactivation by heating of crude extract significantly reduced the cutaneous reaction developed (2.52 ± 0.08 vs 3.54 ± 0.26 , $p < 0.01$), with values of extravasated Evans blue similar to those of saline solution (1.97 ± 0.17).

The toxic mechanism of hairs from caterpillars of different lepidopteras has been demonstrated (Battisti et al., 2011; Fagrell et al., 2008; Lamy et al., 1986; Vega et al., 2011). It mostly involves MCs degranulation with subsequent histamine release and urticaria is one of the main skin reactions associated (Vega et al., 2011). A recent study demonstrates the slow onset of skin lesions after intracutaneous exposure to hairs of the irritating specie *Thaumetopoea pinivora*, contradicting the notion that rapid reactants such as histamine are involved (Battisti et al., 2011; Fagrell et al., 2008). In our study we observed *in vitro* MCs activation and histamine secretion in response to crude extract from hairs of *M. ehrenbergii*. Dermal MCs degranulation in mice is noted in contact with the pine processionary caterpillar, *Thaumetopoea pityocampa* (Kalender et al., 2004). This IgE-independent degranulation has been associated with the protein thaumetopoein (Lamy et al., 1983). We also observed that intradermal injection of crude extract from hairs of *M. ehrenbergii* induces an urticarial reaction in the rat. The temporality of the inflammatory reaction is similar to that developed by histamine or C48/80 injection. Total hair extract from *T. pityocampa* caterpillar causes positive skin reactions when injected

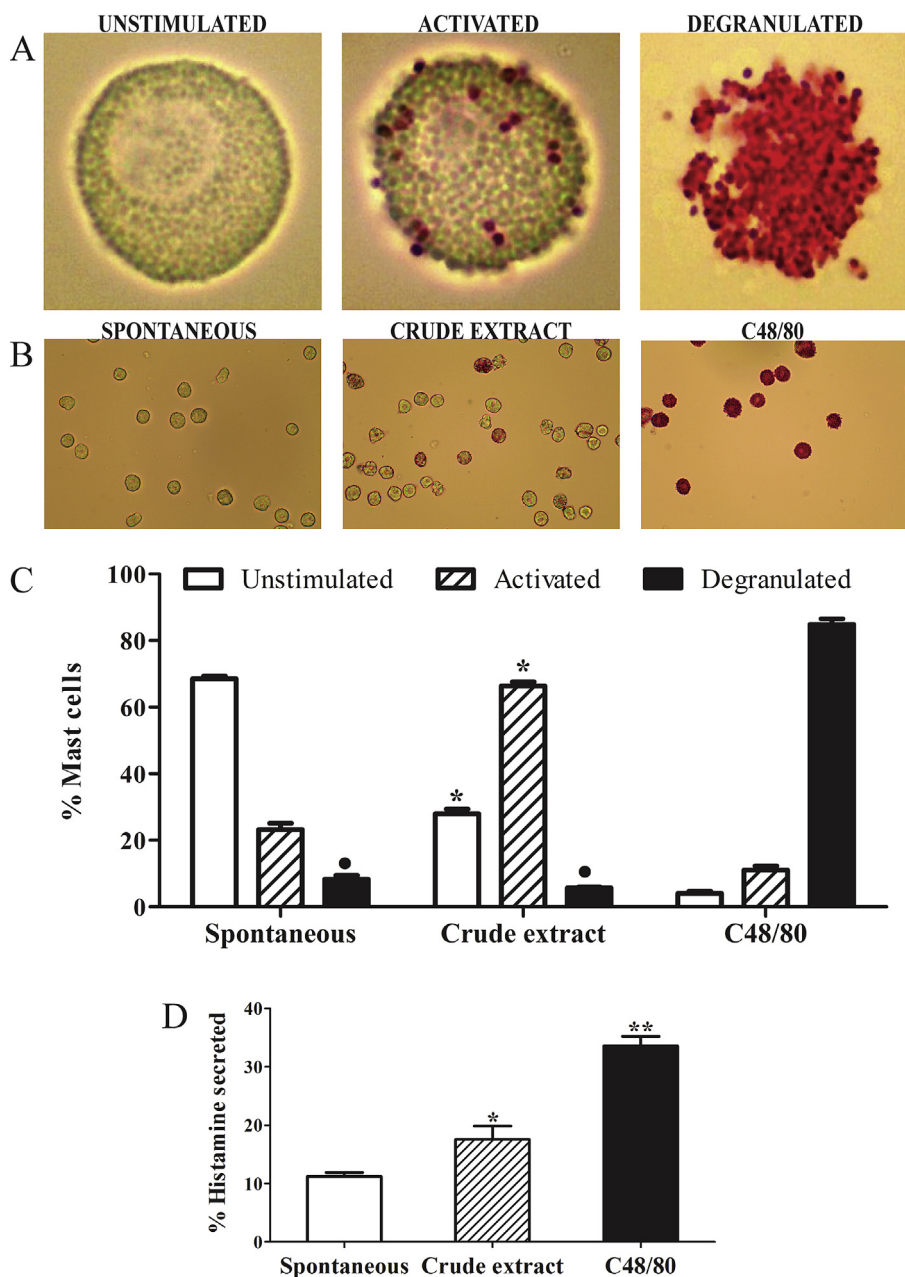


Fig. 1. MCs activation and histamine release. (A) Ruthenium red staining of peritoneal MCs. Activated granules were stained in red and three MCs states were reported: unstimulated, activated and degranulated. (B) Cells were incubated with 0.005% of ruthenium red and crude extract of *M. ehrenbergii* or 5 $\mu\text{g}/\text{ml}$ of C48/80. MCs incubated with 0.005% of ruthenium red alone were representative of spontaneous stimulation. Photographs A were taken at 100 $\times$ and B at 40 $\times$. (C) MCs percentage in each state at spontaneous, crude extract of *M. ehrenbergii* and C48/80 stimulation was counted in a Neubauer chamber at 40 $\times$. Data represents means $\pm$ S.E. of three independent duplicated experiments ($n = 6$). * $p < 0.001$ crude extract of *M. ehrenbergii* versus correspondent state in spontaneous stimulation; • $p < 0.001$ versus degranulated in C48/80 stimulation. (D) Histamine released by MCs following exposure to crude extract of *M. ehrenbergii*. Peritoneal MCs were incubated with supplemented RPMI (spontaneous), crude extract of *M. ehrenbergii* or C48/80 during 30 min. The percentage of histamine released was determined in each case. Values represent the mean $\pm$ S.E. of three independent duplicated experiments ($n = 6$). * $p < 0.01$ crude extract stimulated histamine secretion versus spontaneous; ** $p < 0.001$ C48/80 versus spontaneous and crude extract stimulated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

intradermally in guinea pigs (Lamy et al., 1986). A fraction from this extract containing the protein thaumetopoein induces the same skin reaction as the total extract (Lamy et al., 1985). As crude extract of *M. ehrenbergii* has a pH between 5.0 and 5.5, similar to that of the skin, and it is

isotonic (230 mOsm/L), our results suggest that a substance present in the hairs is involved in skin reaction after caterpillar contact. Heating of crude extract from *M. ehrenbergii* avoid the development of urticarial reaction in the rat skin, indicating that the reaction was not elicited by

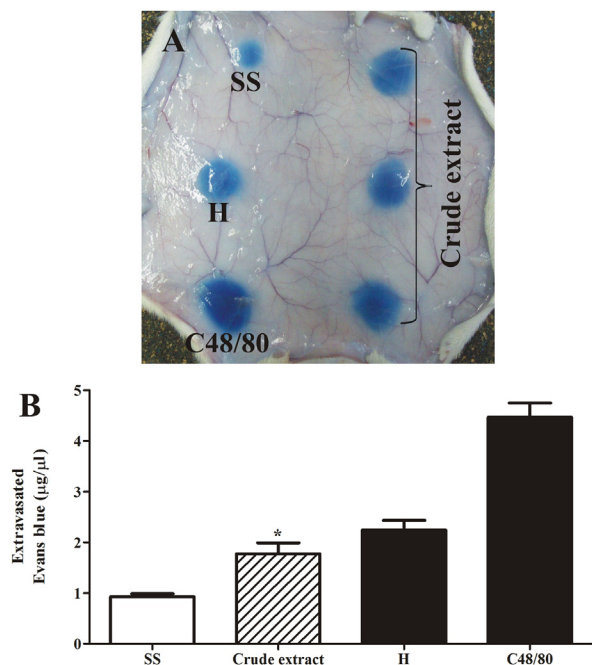


Fig. 2. Cutaneous reaction induced by crude extract of *M. ehrenbergii* (A) Photographs of dorsal inner skin reactions generated by intradermal injection of crude extract of *M. ehrenbergii*. Saline (SS), histamine (H) and C48/80 solutions were used as one negative and two positive controls, respectively. (B). Evaluation of extravasation generated in intradermal skin reactions. The amount of Evans blue extravasated in the skin of rats in response to each external agent was calculated. Values represent the mean $\pm$ S.E. of three independent triplicated experiments ($n = 9$). * $p < 0.05$ crude extract versus ss. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a possible content of histamine in hairs because this pre-treatment does not alter histamine but denatured proteins, and besides histamine was not detected in the crude extract. So, this urticating substance might be a soluble component that builds up the hair or is inside in an internal channel. As there are no reports about systemic toxicity after *M. ehrenbergii* contact, histamine released from MCs may be only participating in urticarial reaction. This data suggests that oral antihistaminic drugs may be used for treatments of skin adverse events as they are used in urticarial reactions generated by other caterpillars (Hossler, 2010b).

In conclusion we found that crude extract of *M. ehrenbergii* hairs induces an urticarial reaction in rat skin. This effect may be associated with MC activation and histamine release and is mediated by a protein component. The characterization of the protein involved in urticarial reaction is the aim of a current investigation.

Ethical statement

The authors declare that all experiments with animals were carried out with strict adherence to ethical guidelines approved by the Institutional Normative Welfare Standards and that the institutional committee for care and use of laboratory animal approved the study.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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