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Dose-dependent effect of histamine on antibody generation *in vivo*

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ABSTRACT

Objective: To delineate immunomodulatory role of histamine on antibody generation profile in rabbit in the present dose-dependent histamine study. **Methods:** The cohort comprised of three groups (III, IV and V), containing six rabbits each, and received subcutaneous histamine 50 μg/kg × bis in die (b.i.d.), 100 μg/kg × b.i.d. and 200 μg/kg × b.i.d., respectively for 10 days (starting from the 1st day). They were subsequently immunized on the 3rd day with intravenous injection of sheep blood cell (SRBC) (1×10^9 cells/mL). Group II (positive control) (n=6) received vehicle (sterile distilled water) and immunized at day 3 similarly while group I (negative control) (n=6) remained non-immunized and received only vehicle. All experimentations were performed in triplicate. Blood samples were collected on pre-immunization (pre-I) (day 0), as well as on days 7-, 14-, 21-, 28- and 58- post-immunization (post-I). Immunological parameters [total immunoglobulins (Igs), IgM and IgG] were analyzed by enzyme linked immunosorbent assay (ELISA) technique. **Results:** Histamine could influence a detectable antibody response to SRBC as early as day 7-post-I, which lasted until day 58- post-I. The results were found statistically significant (P< 0.05). **Conclusions:** Our results provide evidence that histamine has a short-term effect on antibody generation (until its presence in the body), and the antibody generation titer *in vivo* were affected by the concentration of histamine.

1. Introduction

2-(imidazol-4-yl) ethylamine is known as histamine (a biogenic amine), probably one of the most studied molecule in biological system to communicate among cells in different biological activities through differential expression of four types of histamine receptors (H1R, H2R, H3R and H4R) on secretion by effector cells (mast cells and basophils) through various immunological or non-immunological stimuli[1]. Immunosuppressive and immunomodulatory effects of histamine on both humoral- and cell-mediated immune responses have been observed[2,3]. Histamine mediates immediate-type hypersensitivity with increased vascular permeability, smooth muscle contraction, vasodilatation, flushing, mucus secretion, and pruritus[4,5], and also have been shown to enhance delayed hypersensitivity and antibody mediated immune responses[6-8]. Its broadest spectrum of activities have been identified by histamine receptors in various physiological and pathological conditions including the cell proliferation, differentiation, hematopoiesis, embryonic development, regeneration, wound healing, aminergic neurotransmission and numerous brain functions, secretion of pituitary hormones, regulation of gastrointestinal and circulatory functions, cardiovascular system, as well as inflammatory reactions, modulation of the immune response, energy of endocrine and homeostasis[1]. However, studies evaluating the role of histamine, especially studying the immunomodulatory profile over a span of time are lacking in the existing literature.

The present study was therefore designed to delineate the dose-dependent effect of histamine in immunomodulation specially the immunoglobulins [total immunoglobulins (Igs), IgG and IgM] generation profile over a period of 58 days in rabbit model.

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2. Materials and methods

2.1. Experimental design

To evaluate the systemic antibody response, New Zealand adult healthy albino rabbits of either sex (1 ∶ 1 ratio) weighing 1.0–1.5 kg were divided into five treatment groups. Each group contained six rabbits. Four groups were immunized with sheep red blood cells (SRBC).

Experimental groups including Groups III, IV and V were treated with histamine respectively for 10 days (starting from the 1st day).

Control group, group–II (positive control) received vehicle (sterile distilled water) and immunized while group–I (negative control) remained non–immunized and received only vehicle.

They were housed in well–maintained animal facility at Central Animal House, J. N. Medical College, Aligarh Muslim University, Aligarh, in the Bioresources unit under a 12 h light/dark cycle, temperature [(22±2) ℃] and were allowed free access to standard laboratory diet including green vegetables and tap water until experimentation. All studies were carried out during the light cycle and were approved by the Institutional Animal Ethical Committee.

2.2.Materials

All materials were obtained from the following manufacturers: Monoclonal–anti–rabbit–immunoglobulins–horseradish peroxidase (HRP) conjugate and monoclonal–anti–rabbit–IgG–HRP conjugate from Sigma (USA), anti–rabbit–IgM–HRP conjugate from G Biosciences from Maryland heights (USA), tetramethyl benzidine (TMB) and TMB diluent from J. Mitra and Co. (India), Polystyrene MaxiSorp microtitre flat bottom enzyme linked immunosorbent assay (ELISA) plates from NUNC (Denmark), Glutaraldehyde solution from Central Drug House (India), Skimmed milk from Nestle India Ltd. (New Delhi). All chemicals were of analytical grade.

2.3.Drug and dose

Histamine dihydrochloride was purchased from Himedia laboratories Pvt Limited, India. The dose were 50 μg/kg, 100 μg/kg and 200 μg/kg in groups III, IV and V, respectively, and was administered twice in a day through subcutaneous (s.c.) injection. Histamine dose is referred to the weight of the salts used.

2.4.Antigen

Sheep blood diluted at 1 ∶ 1 in sterile Alsevier′s solution was obtained from Department of Microbiology, J. N. Medical College, A.M.U., Aligarh, and washed with phosphate buffered saline (PBS) (10 mM sodium phosphate buffer containing 150 mM NaCl, pH–7.4) thrice by centrifugation. The cell suspensions were adjusted to the desired concentration in terms of hemoglobin, lysis of a 1% SRBC suspension (2×10^8 cells/mL) with 14 volumes of 0.1% Na_2CO_3 develops an optical density of 0.135 at 541 nm in a spectrophotometer (Systronics, UV visible double beam spectrophotometer–2101, India), as described by Franzl[9]. Finally the concentration was adjusted to 5% (1×10^9 cells/mL) in PBS for immunization before use.

2.5.Immunization of rabbits

The rabbits in experimental groups (II–V) were immunized on the 3rd day intravenously (i.v.) with 1 mL of 5% (1×10^9 cells/mL) SRBC in PBS.

2.6.Sample collection

To determine the systemic antibody response, blood samples were collected from rabbits through the marginal ear veins prior to immunization (day 0), as well as on days 7–, 14–, 21–, 28– and 58– post–I. Blood samples were kept at room temperature for 2 h and then at 4 ℃ overnight. Blood samples were centrifuged for 10 minutes at 580× g, and serum was separated and heated at 56 ℃ for 30 minutes to inactivate complement proteins and stored in aliquots containing sodium azide as preservative at –20 ℃ till further testings.

2.7.Serological method

To determine the SRBC–specific–immunoglobulins (Igs), SRBC–specific–IgM and SRBC–specific–IgG response, the whole SRBC– ELISA[10] was carried out on polystyrene plates with some modifications. Briefly, polystyrene MaxiSorp immunoplates were coated with SRBC suspension [5×10^6/100 μL PBS (10 mM sodium phosphate buffer containing 150 mM NaCl, pH–7.4)]. The plates were held overnight at 4 ℃. Each sample was coated in duplicate and half of the plates served as control devoid of antigen coating. Without disturbing the cell layer, 20 μL of 1.8% glutaraldehyde solution was then gently added to plates inoculated with SRBC and the plates were held at 25 ℃ for 30 mins. Unbound SRBC was washed four times with 200 μL of PBS and non–specific binding sites were blocked with 1% fat–free milk in PBS for 2 hrs at 37℃. After incubation, the plates were washed four times with 200 μL of PBS. Each rabbit serum diluted at 1 ∶ 100 in PBS (100 mL/well) was adsorbed for 1.5 hrs at 37 ℃, and then overnight at 4 ℃ followed by washing as earlier. The secondary antibody, HRP conjugated monoclonal–anti–rabbit–immunoglobulins, monoclonal–anti–rabbit–IgM and monoclonal–anti–rabbit–IgG was then added (100 μL/well) in respective plates and incubated at 37 ℃ for 1 hr. The washing stage was repeated as before and 100 μL/well TMB substrate was added and the plates were incubated at 25 ℃ for 1 hr. The enzymatic reaction was stopped by adding 50 μL/well of 5% H_2SO_4. The absorbance (A) was determined at 405 nm on an automatic ELISA plate reader (Micro scan MS5608A, ECIL, India). Each rabbit serum sample was run in duplicate. The control wells were treated similarly but were devoid of antigen. Results were expressed as a mean of $A_{test-control}$.

2.8.Statistical analysis

Data were presented as mean±SD of triplicate experiments. Significance of differences from control values were determined with the paired student′s *t*–test (SPSS 12.0 for windows, Inc., Chicago, IL). Statistical significance was concluded at $P<0.05$.

3. Results

To assess the effects of histamine on the antibody generation, antibody–mediated responses to SRBC were analyzed. Total serum immunoglobulins, immunoglobulin–M (IgM) and immunoglobulin–G (IgG) generation profiles were studied *in vivo* in five experimental groups (in three separate experimentations) at days 0 [pre–immunization (pre–I)], 7, 14, 21, 28 and 58 [post–immunization (post–I)].

3.1.Total serum anti–SRBC–antibody production

The profile of total anti-SRBC-antibody titer was studied by whole SRBC-ELISA method[10] (Figure 1). No antibody response was detected in any experimental groups at day 0 (pre-I). In positive control group antibody titer showed an initial increase on day 7- post-I, which achieved highest peak at day 14- post-I and then a gradual decrease in anti-SRBC-antibody titer over time (day 21-, 28- and 58- post-I). Histamine treated groups III, IV and V showed different patterns in total antibody titer at day 7-, 14-, 21-, 28- and 58- post-I. All the histamine treated groups (III, IV and V) showed enhanced antibody titer at day 7- post-I as compared to positive control (group II). While group V showed significant enhancement of total antibody titer at days 7-, 14-, 21-, 28- and 58- post-I ($P< 0.000\,1$) as compared to rest of the groups (II, III and IV). Group IV revealed significant ($P<0.05$) enhancement of total antibody titer as compared to group II, while it showed insignificant enhanced total antibody titer as compared to group III, at day 7- post-I. However, group III revealed insignificant increased total antibody titer at day 7- post-I as compared to group II. Moreover, total antibody titer at days 14-, 21-, 28- and 58- post-I in group III and IV showed insignificant suppressed total antibody titer as compared to group II. No antibody response was noticed in group I (negative control) during whole of the study period.

3.2.Anti-SRBC-IgM production

Anti-SRBC-IgM was determined by whole SRBC-ELISA method[10] (Figure 2). No antibody response was detected in any experimental group (negative control, positive control or histamine-treated) at day 0 (pre-I). However, there was an initial increase and then gradual decrease in anti-SRBC-IgM titer over time in all the groups. By day 7- post-I, the IgM titer increased and obtained highest peak, but by days 14-, 21-, 28- and 58- post-I there was a gradual decrease in all experimental groups. Group V showed significant ($P<0.000\,1$) enhancement of anti-SRBC-IgM titer at day 7-, 14-, 21-, 28-, 58- post-I as compared to other experimental groups (II, III and IV). However, group IV showed insignificant enhancement (at day 7- post-I) and suppression (at day 14-, 21-, 28- and 58- post-I) of anti-SRBC-IgM titer as compared to group II. Moreover, group IV revealed significant enhanced anti-SRBC-IgM titer at day 7- post-I, while at day 14-, 21-, 28- and 58- post-I, it showed insignificant difference of anti-SRBC-IgM titer to group III. Group III revealed significant suppression of anti-SRBC-IgM titer at day 7-, 14- and 21- post-I as compared to group II. However, at day 28- and 58- post-I, anti-SRBC-IgM titer was same in group II, III and IV. No antibody response was noticed in group I (negative control) during whole of the study period.

3.3.Anti-SRBC IgG production

Anti-SRBC-IgG was determined by whole SRBC-ELISA method[10] (Figure 3). No antibody response was detected in any experimental groups at day 0 (pre-I). By day 7- post-I, the IgG titer increased but it obtained highest peak at day 14- post-I, while by days 21-, 28- and 58- post-I it gradually fell down or maintained a plateau in the group II (positive control). However, IgG titer in histamine-treated groups (III and IV) revealed significant ($P<0.05$) enhancement as compared to group II at day 7- post-I. However, IgG titer in group IV showed insignificant suppression, while, in group III it was significantly ($P<0.05$) suppressed, at day 14-, 21-, 28- and 58- post-I as compared to group II. IgG titer in group III was insignificantly suppressed to the IgG titer of group IV over a span of study period. Moreover, group V showed high significant ($P<0.000\,1$) enhancement of anti-SRBC-IgG titer at days 7-, 14-, 21-, 28- and 58- post-I as compared to rest of the experimental group (II, III and IV). No antibody response was noticed in group V (negative control) during whole of the study period.

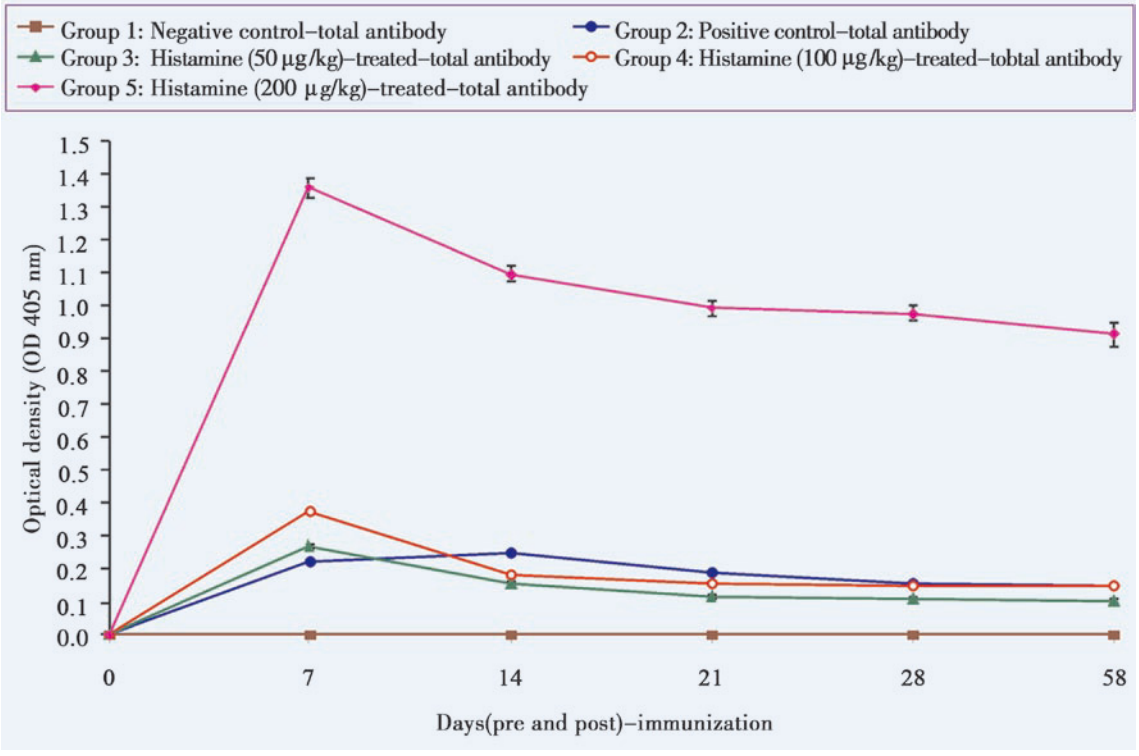

Figure 1. SRBC-specific immunoglobulins (Igs) production in rabbits by whole SRBC-ELISA method in duplicate 1 : 100 diluted sera.

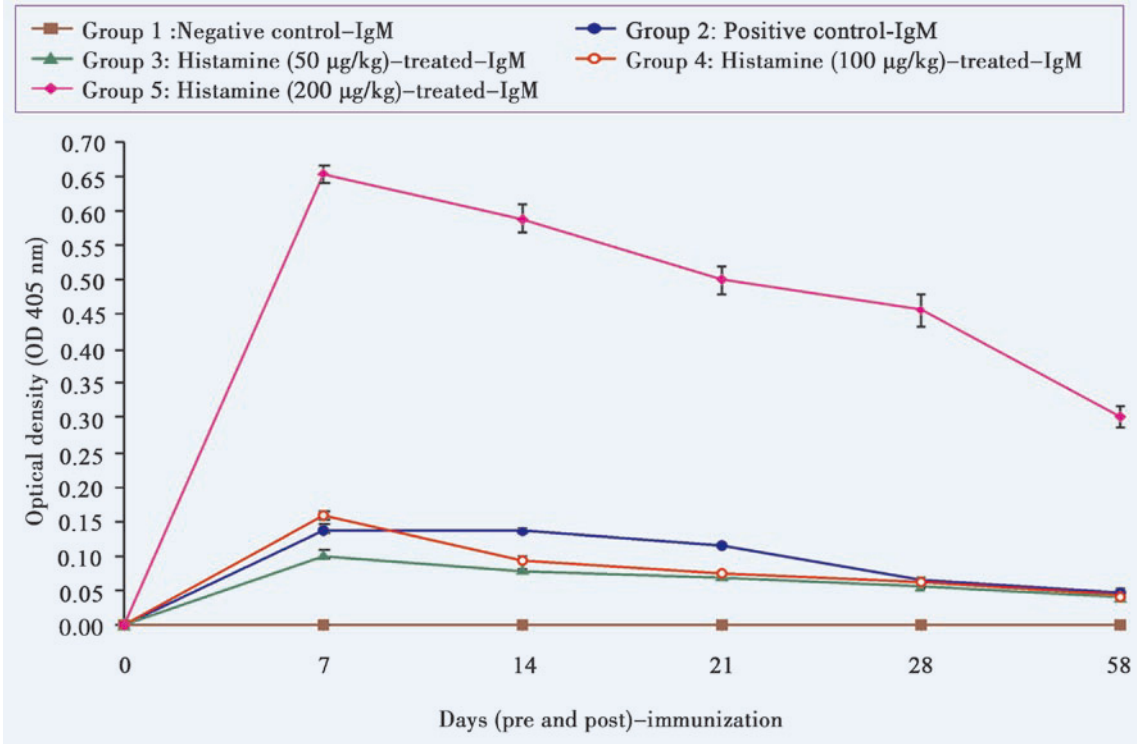

Figure 2. SRBC-specific immunoglobulin-M (IgM) production in rabbits by whole SRBC-ELISA method in duplicate 1 : 100 diluted sera.

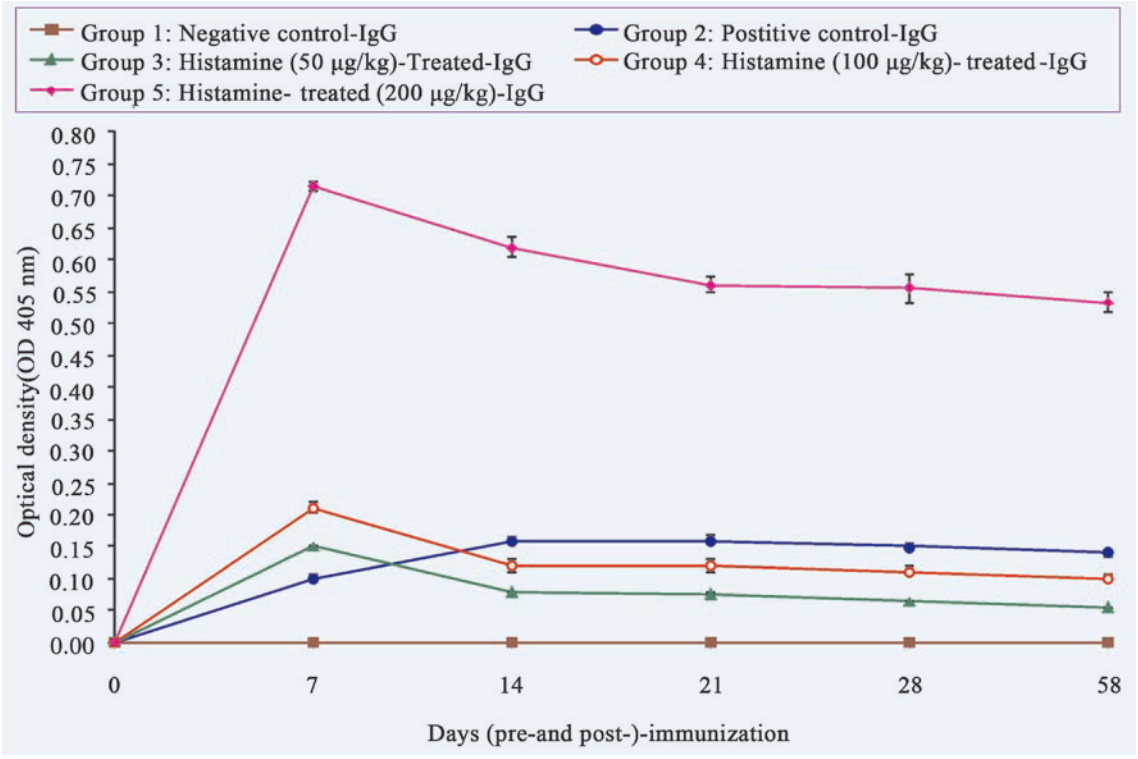

Figure 3. SRBC-specific immunoglobulin-G (IgG) production in rabbits by whole SRBC-ELISA method in duplicate 1 : 100 diluted sera.

4. Discussion

In the present report, we studied the effect of histamine as an immunopotentiating agent via the dose-dependent experimentations in albino rabbits and demonstrated the effect of histamine on antibody generation profile.

We investigated total serum immunoglobulins, IgM and IgG generation profile against SRBC, a T lymphocyte-dependent test antigen[10], modulated by endogenous histamine (released from effector cells) in positive control (sterile distilled water-treated)- and histamine treated-experimental groups. According to the document of International Conference on Harmonization (ICH) S8 Guideline on Immunotoxicity Testing for Pharmaceuticals (adapted by EU in 2005, and by the FDA and MHLW in 2006), the evaluation of antibody response to a

T-lymphocyte-dependent antigen (e.g., SRBC or Keyhole limpet hemocyanin) is recommended as one of the most sensitive immune tests following chemical exposures[10]. The T-lymphocyte-dependent antibody response assay is a sensitive indicator of immunological integrity. When SRBC is used as the particular T-lymphocyte-dependent antigen, this response requires the coordinated interaction of various immune system cells (i.e., antigen-presenting cells, T-lymphocytes and B-lymphocytes)[10,11]. Therefore, we hypothesized the present work to delineate the immunomodulatory role of histamine against SRBC.

Based on the present study, we demonstrate that the histamine, released from immunological stimuli from effector cells (mast cells and basophils) *in vivo*[1], could influence a detectable antibody response to SRBC as early as day 7- post-I, which lasted until day 58- post-I in all experimental groups. Histamine, itself is a potent agonist of all four receptors (H1, H2, H3 and H4)[1,3,12], which are distributed in different parts of body and modulates different biochemical, pharmacological and immunological reactions both *in vivo* and *in vitro* by the activation of signal transduction on releasing the endogenous histamine[1]. It is documented that histamine directly effects B-cell antibody production as a co-stimulatory receptor on B-cells[13-16]. It must be emphasized here that, to the best of our knowledge, none of the earlier reports have shown the anti-SRBC antibody profile over a span of study period (58 days) (except our earlier report on immunomodulatory profile during a study period of 28 days)[17], rather all previous reports have studied the anti-SRBC antibody concentrations at a single time period taking single blood samples from experimental animals. We demonstrated that the histamine treated-rabbits showed immunopotentiating properties by enhancing the anti-SRBC-antibodies (total antibody, IgM and IgG) titers as compared to positive control group over a span of study period.

In this preliminary study, we noticed immunomodulatory effect of histamine at three different doses (50 μg/kg, 100 μg/kg and 200 μg/kg), and observed their outcome on antibody generation titer. It was interestingly noticed that 50 μg histamine-treated group affect total antibody and IgG generation titer at day 7- post-I (until histamine presence in the body). However, 100 μg and 200 μg histamine-treated group at day 7- post-I (until its presence in the body) enhanced total antibody, IgM and IgG generation titer whereas; on further metabolism histamine effect disappeared on the antibody generation profile bringing it in the range of control antibody level in 50 μg and 100 μg histamine-treated group, while it was disappeared in 200 μg histamine-treated group. Therefore, on the basis of the present study we reached to a conclusion that the results obtained due to histamine were of short duration which disappeared in latter stage of 50 μg and 100 μg histamine-treated groups due to clearance of the body from these substance in rabbits.

The results provide evidence that antibody generation titer *in vivo* affected by the concentration of histamine. This study would definitely stimulate for active discussions and warrants further researches on this important aspect including histamine-role on antibody generation mechanism in immune modulation and regulation. To the best of our knowledge, this is the first report describing the potential immunoregulatory role of histamine dose-dependently.

Conflict of interest statement

We declare that we have no conflict of interest.

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