Research Article

Evaluation of Immunomodulatory Activity of H2-Antagonists Like Ranitidine And Famotidin In Mice

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ABSTRACT

To evaluate Ranitidine and Famotidine for their Innate immunomodulatory activity SRBC antigen challenge, Carbon Clearance and Cecal Ligation and Puncture tests were performed, Swiss albino mice were administered Ranitidine 62.5 mg/kg, i.p, and Famotidine 80 mg/kg, p.o. doses daily for 7 days. 3 hours after the last dose all the animals of each group was given colloidal carbon intravenously in a volume of 1ml/100g. Blood samples were then collected (25 µl) from retro orbital plexus at 0 and 30 minutes after injection of colloidal carbon ink and lysed in distilled water (3ml). The optical density was measured spectrophotometrically at 650 nm. For Cecal Ligation and Puncture animals received drugs 18 hrs before CLP and % survival was measured for 7 days after CLP.

The Ranitidine 62.5 mg/kg, i.p, and Famotidine 80 mg/kg, p.o, stimulated primary and secondary humoral immune response ($p<0.001$), Phagocytic index in Carbon clearance ($p<0.01$) and H_2-blockers treated group significantly increased percentage survival at 24,48 up to 168hr as compare to control group.

The H2-antagonists appears to have significant immunostimulant property in mice at the specified doses. In addition, it was found that these agents could be used successfully and safely for the short duration of treatment. Thus, Ranitidine and Famotidine can be used as an adjuvant in many cases like cancers (e.g. colorectal, breast, prostate etc.), HIV infection and immunocompromised conditions.

Keywords: immunomodulatory activity, Ranitidine, Famotidine.

1. INTRODUCTION

Histamine is an important inhibitor of the production of IL-12 by monocytes. Although SAC is an activator of both B cells and monocytes, B cells hardly contribute to the production of IL-12. In whole blood cultures, monocytes are considered the main IL-12- and IL-6- producing cells, because CD14-depleted MNC or granulocytes did not produce IL-12 or IL-6 after stimulation with SAC. In agreement with a direct inhibitive effect of histamine on the production of TNF-α and IL-1 in isolated monocytes[1]. It has been observed that of IL-12 production was inhibited by histamine in isolated monocytes, which could be reversed by an H_2 antagonist.Histamine (through interaction with the H_2 receptor) activate adenylate cyclase, which leads to the generation of increased levels of cAMP in various cell types[2]. Upon incubation with histamine cAMP level was increased in purified monocytes, which can be prevented by an H_2 antagonist. Because both the inhibition of IL-12 production and enhancement of cAMP levels by histamine were mediated through the H_2 receptor and the effects of histamine on IL-12 production were additive, histamine most likely inhibit IL-12 production by a similar cAMP dependent mechanism. The exact pathway of cAMP-induced inhibition of IL-12 production is presently unknown. In T cells, a cAMP-inducible transcriptional repressor protein (ICER, inducible cAMP early repressor) has been described to inhibit IL-2 promoter activity[3]. This mechanism probably involves binding of ICER to cAMP responsive elements (CRE), thereby inhibiting transactivating CRE-binding proteins. A similar mechanism may be responsible for the cAMP-induced inhibition of

IL-12 production, since the human IL-12 p40 promoter contains a CRE-like motif. Another explanation may be reduced activation of NF-kB through cAMP-induced retardation of degradation of the inhibitor of NF-kB, IkBa[4]. Murine p40 transcription has been shown to be positively regulated by NF-kB[5]. Because the human IL-12 p40 promoter contains a NF-kB site[5].this may also be true for human p40 regulation.

Histamine is associated with Th2-mediated (allergic) diseases.Histamine is released during allergic reactions after interaction of allergens with cell-bound IgE on basophils and mast cells.In addition, PBMCs from atopic dermatitis patients show enhanced basal activation of PKA and increased levels of cAMP. In allergic asthma patients with enhanced IgE levels, reduced capacity to produce IL-12 p70 in response to SAC was found. The in vivo exposure of monocytes to histamine could have contributed to this. Similar observations were reported on HIV-infected patients.

Overproduction of histamine can lead to Th2 responses by several mechanisms. Histamine (H_2 receptormediated) inhibits TNF-a production by monocytes. TNF-α may contribute to Th1 development by potentiating IL-12 induced production of IFN-γ in PBMCs. In addition, cAMP and as result histamine inhibit secretion of the Th1 cytokines IL-2 and IFN-γ but not the Th2 cytokines IL-4 and IL-5[6,7]. However, the most important mechanism of induction of Th2 development is probably the inhibition of IL-12 production, because the concentrations of histamine that inhibit the production of IL-12 is approximately100-fold lower,than those required for inhibition of T cell cytokine production. The levels of histamine that completely inhibit the production of IL-12 are well within the physiological range. Histamine reaches levels as high as 10^{-7} M in serum or nasal washing fluids, suggesting that concentrations of histamine in tissue may even be higher. Initial histamine release is dependent on the presence of IgE, induced by Th2 cytokine production. Histamine may provide a positive feedback on continued Th2 differentiation by the inhibition of IL-12. In addition, histamine enhances anti-CD58 plus IL-4 or IL-13 induced IgE production in purified B cells.[8] Interestingly, a positive feedback loop of histamine on Th2 differentiation appears to be more evident in allergic patients.

The observation that the cAMP/PKA pathway (possibly induced by histamine) is overactive in a number of pathological conditions such as HIV infection and atopic diseases (Such as asthma) may lead to new intervention strategies designed to up regulate IL-12 production. The use of inhibitors of histamine H_2 antagonists may be valuable to redirect Th2 to Th0/Th1 responses in these conditions and may be combined with vaccination or hypo sensitization therapy.

Hence, the present study is aimed for evaluating immunomodulatory activity of few histamine H_2-antagonists, which belongs to the category of antiulcer.

2. MATERIALS AND METHODS

2.1 Mice

The Institutional Animal Ethics Committee has approved the animal studies. Swiss albino mice of inbred colony obtained from National Toxicology Center, Pune, of either sex weighing 20-25 gm were housed in groups of 5 to 6. Mice were maintained at standard laboratory conditions. All mice were fed with synthetic pelleted diet, (Amrut laboratory animal feed, Sangli-Maharashtra) and clean tap water ad libitum. Mice were maintained at 22ºC ± 1ºC with 60% relative humidity and kept under 12 h light:dark cycle. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. All experiments were conducted during the light period of 12/12 hours of the day/night cycle.

2.2 Drugs and Chemicals

Ranitidine was gifted by Zydus-Cadila Pvt Ltd,Famotidine was gifted by U.S.Vitamins Pvt.Ltd.All other reagents used were of analytical grade.

2.3 Treatment

For the experimental group was divided into various subgroups (n=6) and received a dose of Ranitidine 62.5 mg/kg, i.p, and Famotidine 80 mg/kg, p.o., once daily from 1st to 21st day, while the control group received only vehicle. For Carbon Clearance test the immunomodulatory activity of H_2-antagonists were given daily for 7 days.And for Cecal Ligation And Puncture test the H_2-antagonists were given once, 18 hours before CLP .

2.4 Immunological response

2.4.1 Humoral immune response[9]

On 14th and 21st day, blood was withdrawn from the retro-orbital plexus of all antigenically challenged mice. 25 µl of serum was serially diluted with 25 µl of phosphate-buffered saline. SRBC (0.025×10^9 cells) were added to each of these dilutions and incubated at 37°C for one hour. The rank of minimum dilution that exhibited hemagglutination was considered as an antibody titer. The level of antibody titer on 14th day of the experiment was considered as

the primary humoral immune response and the one on 21st day of the experiment was considered as the secondary humoral immune response[9].Results were expressed as mean ± S.E.M. and statistical significance on primary and secondary antibody titer was assessed by means of one-way ANOVA followed by Tukey-Kramar multiple comparison tests.

2.4.2 Carbon Clearance test[10]

The animals were divided into 4 groups consisting 5 animals each. All the groups were treated with their respective treatment as described above. On day 7, 3 hours after the last dose all the animals of each group was given colloidal carbon intavenously in a volume of 1ml/100g. Blood samples were then collected (25 µl) from retro orbital plexus at 0 and 30 minutes after injection of colloidal carbon ink and lysed in distilled water (3ml). The optical density was measured spectrophotometrically at 650 nm .The phagocytic index was calculated from the following equation

$$K=\frac{(\text{In OD1} - \text{In OD2})}{(t2 - t1)}$$

Where OD1 and OD2 are the optical density at time t1 and t2 respectively.

The results were expressed as mean ± S.E.M. Data on prcentage change in foot paw edema were analyzed by one-way ANOVA followed by Tukey-Kramar multiple comparison test.

2.4.3 Cecal ligation and puncture induced abdominal peritonitis[11]

Mice were aneshetised by Ketamine 100 mg/kg ,i.p. A 1–2cm midline incision was made through the abdominal wall (Fig 5.1a); the cecum was identified and ligated with a 3-0 silk tie 1 cm from the tip (Fig 5.1b). Care was taken not to cause bowel obstruction. A single puncture of the cecal wall was performed with a 20-gauge needle. The cecum was lightly squeezed to express a small amount of stool from the puncture site in order to assure a full thickness perforation. The cecum was returned to the abdominal cavity and the incision was closed with surgiclips (Fig 5.1c). Sham mice underwent anesthesia and midline laparotomy; the cecum was exteriorized, returned to the abdomen and the wound was closed with surgiclips. Measurement of mortality was carried out for 7 days after CLP.

3. RESULT

3.1 Humoral immune response

3.1.1Primary Humoral Immune Response

At the end of 14 days prophylactic treatment of Ranitidine, Famotidine and Salbutamol, the animals of each group were subjected to primary and secondary antigen challenge by Sheep RBCs. Primary antibody titer was determined and shown in the Table No.1.

Mice in the cyclophosphamide control group had shown significantly less antibody titer (***p< 0.001) when compared to control. In the group of mice with normal immune status, Ranitidine showed significant (*p<0.05) rise in primary antibody titer, while Famotidine did not produce significant rise in primary antibody titer. Where as in the groups of mice treated with cyclophosphamide *i.e*. immunosuppresed, groups treated with Ranitidine (***p<0.001) and Famotidine (***p<0.001), significantly raised primary antibody titer.

Ranitidine (62.5 mg/kg. i.p.) in combination with cyclophosphamide *i.e*. an immunosuppresed Ranitidine treated group enhanced the primary antibody titer significantly (***p<0.001) when compared with cyclophosphamide control.

Famotidine (80 mg/kg. p.o.) in combination with cyclophosphamide *i.e*. an immunosuppresed Famotidine treated group enhanced the primary antibody titer significantly (***p<0.001) when compared with cyclophosphamide control.

3.1.2Secondary Humoral Immune Response

At the end of 14 days prophylactic treatment of Ranitidine and Famotidine, the animals of each group were subjected to antigen primary and secondary antigen challenge by sheep RBCs. Secondary antibody titer is determined and is shown in the Table No 2.

Mice in the cyclophosphamide control group had shown significantly less antibody titer (**p<0.01) when compared to control. In the group of mice with normal immune status, Ranitidine (***p<0.001) and Famotidine (***p<0.001) produced significant rise in secondary antibody titer. Groups treated with two different H_2-blocker drugs, significantly raised secondary antibody titer when compared with cyclophosphamide control.

Ranitidine (62.5 mg/kg. i.p.) in combination with cyclophosphamide *i.e*. an immunosuppresed Ranitidine treated group enhanced the secondary antibody titer significantly (**p< 0.01) when compared with cyclophosphamide control.

Famotidine (80 mg/kg. p.o.) in combination with cyclophosphamide *i.e*. an immunosuppresed Famotidine treated group enhanced the secondary antibody titer

significantly (**p< 0.01) when compared with cyclophosphamide control.

3.2 Carbon Clearance Test

To assess the functional changes in, macrophages of reticuloenothelial system, their phagocytic ability was determined. Table No.3 shows the effect of some H_2-antagonists on Reticulo-Endothelial System by Carbon Clearance Test.

Ranitidine treated group, at a dose of 62.5 mg/kg exhibited significantly high phagocytic index of 0.0336.

Similarly 80 mg /kg dose of Famotidine treated group exhibited significantly high phagocytic index of 0.0248.

3.3 Cecal Ligation and Puncture induced abdominal peritonitis

In Cecal Ligation and Puncture induced abdominal peritonitis model, percentage survival due to polymicrobial sepsis was recorded. Table No. 4 summarizes the effect of Ranitidine and Famotidine in Cecal Ligation and Puncture Induced abdominal peritonitis. H_2-blocker treated group significantly increased percentage survival at 24,48 up to 168hr as compare to control group.

After 24hr of CLP the percentage survival in Ranitidine treated group at the dose of 62.5 mg/kg (i.p.) was 100% as against 53% survival in the control group. After 48hr percentage survival was 93.3% against 6.6% in the control group while after 168hrs it was 26.6% against 0% in control group.

After 24hr of CLP the percentage survival in Famotidine treated group at the dose of 80 mg/kg (p.o.) was 86.6% as against 53% survival in the control group. After 48hr percentage survival was 80% against 6.6% in the control group while after 168hrs it was 26.6% against 0% in control group. In the Laparotomy control group, percentage survival was 100% after 168hrs.

4. Tables and Graphs

Table-1 Immunosuppressed drug Treated groups were compared with cyclophosphamide control.
Cyclophosphamide control compared with control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Table-2 Immunosuppressed drug Treated groups were compared with cyclophosphamide control.
Cyclophosphamide control compared with control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Table-3 Drug treated groups were compared with Control.
(Statistically analysed by one-way ANOVA followed by Turky-Kramar multiple comparisons test.)

Table-4 Drug treated groups were compared with Control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

5. Graphs and Figures

Fig 5.1- Procedure for cecal ligation and puncture

Graph-1 Immunosuppressed Drug Treated groups were compared with cyclophosphamide control. Cyclophosphamide control was compared with Control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Graph-2 Immunosuppressed Drug Treated groups were compared with cyclophosphamide control. Cyclophosphamide control was compared with Control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Graph-3 Drug treated groups were compared with Control. (Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Graph-4 Drug treated groups were compared with Control. (Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

6. DISCUSSION AND CONCLUSION

Histamine is an endogenous mediator of many (patho)-physiological processes, such as the regulation of gastric acid secretion and cardiac output, while functioning as a neurotransmitter in the brain. During allergic reactions, histamine is released in large quantities from intracellular stores from mast cells and basophils after cross-linking of cell surface IgE by allergens. Histamine possessing immunomodulatory activity through H_2 receptor on dendritic cells (DCs)[12].DCs display a functional plasticity and can be instructed to polarize T-cells by mediators that are present in the peripheral environment[13].These factors affect the differentiation process of the immature dendritic cells (iDCs), the exit of the mature DCs (mDCs) from the inflamed tissues, and the production of cytokines by mDCs after they have migrated to the lymph nodes.

Intracellular cAMP is an important physiological mediator of the inflammation process, and it also plays a central role in the regulation of the immune response[14]. Physiological and pharmacological agents that increase cAMP levels, such as histamine, β_2-adrenergic receptor agonists and bacterial toxins are able to interfere with the differentiation process of DCs through modulating functional plasticity of iDCs by inhibiting Src-like Kinase through G protein receptor mediated protein kinase A signaling[15].These agents display different effects on the maturation process of iDCs and share the capability of blocking the release of pro-inflammatory cytokines, including IL-12 and TNF-α, as well as stimulating IL-10 secretion by cAMP-dependent or independent mechanisms. H_2 receptors are G-protein couple receptors. H_2 receptor stimulation results in cAMP generation[2].

The induction of a cell-mediated immune response to a specific antigen is regulated, for the most part, by the release of cytokines. IL-12 is produced by macrophages, monocytes, dendritic cells and B cells in response to bacterial products and intracellular parasites[16].The biological effects of the production of IL-12 are directed at T cells and NK cells. IL-12 is responsible primarily for the subsequent production of IFNγ and tumor necrosis factor-alpha (TNF-a) from both NK cells and helper T cells. IL-12 also stimulates the rate at which NK cells and helper T cells proliferate following antigen activation. In addition, the lytic capacities of both NK and helper T cells are increased by the presence of IL-12. IL-12 has the specialized function of leading naive CD4+ T cells to differentiate toward the TH1 cell type in order to prepare for the release of IFN-gamma and for the development of the cell-mediated immune response. However, IL-12 is not effective in the down regulation by means of reversing TH2 cells differentiation. IL-12 and IL-2 are both important cytokines in the regulation of a cell-mediated immune response, IL-2 being responsible for stimulating the growth and proliferation of T cells, while IL-12 stimulates the differentiation of the CD4+ T cells into TH1 cells.

IL-10 was first recognized for its inhibition of T cell activation and effector functions. IL-10 was initially characterized as a cytokine produced by certain Th2 cell clones. Many other cell types, however, such as macrophages, B cells, mast cells, and keratinocytes have been shown to secrete considerable amounts of this cytokine. IL-10 downregulates the expression of MHC class II and costimulatory molecules and inhibits the production of proinflammatory cytokines, including IL-12 by DCs and other professional antigen-presenting cells.

H_2 antagonists increase the level of IL-12 and IL-2, on the other side reducing the level of IL-10 by decreasing the level of cAMP in dendritic cell and ultimately activate immune system.

When mice were sensitized with SRBC, an antigen gets diffused in the extra vascular space and enters the lymph node via the lymphatics. Particulate antigens are taken up by macrophages lining the sinuses or disperse in the lymphoid tissues and processed. Small highly antigenic peptides are combined with MHC class II molecule. B cells with receptors for antigen binds and internalizes it into an endosomal compartment and process and presents it on MHC class II molecules to T_{H2} cells. These B cells are triggered to proliferate, giving rise to clones of large numbers of daughter cells. Some of the cells of these expanding clones serve as memory cells, other differentiates and become plasma cells that make and secrete large quantities of specific antibody. During a primary response, IgM is secreted initially, often followed by a switch to an increasing proportion of IgG. The magnitude of secondary antibody response to the same antigen is amplified in terms of antibody production[17].

In the present study, the assessment of humoral immunity was carried out using hemagglutination test. The anti-SRBC antibody titer in Ranitidine and Famotidine treated groups in the dose of 62.5 mg/kg, 80 mg /kg respectively, with normal immune status, was increased but statistically non significant.

In the immunosuppessed groups Cyclophosphamide was used as immunosuppressant as, it selectively suppresses humoral immunity by exerting depressive effect on antibody production, if given after antigenic stimulation. This may be due to interference with helper T cell activity.

In the immunosuppessed groups, H_2-antagonist significantly protected Cyclophosphamide induced suppression of humoral immunity indicating that H_2-antagonist counteracts suppression of both, primary and secondary humoral immune response induced by cyclophosphamide. This suggests that H_2-antagonist exerts immunoprotective and strong immunomodulatory property. H_2-antagonist stimulates humoral immune response in terms of elevation in anti SRBC antibody titer.

When activated T_{H1} cells encounter certain antigens, *viz* SRBC. They secrete cytokines

that induce a localised inflammatory reaction called Delayed Type Hypersensitivity. DTH comprises of two phases, an initial sensitisation phase after the primary contact with SRBC antigen. During this period T_{H1} cells are activated and clonally expanded by APC with class II MHC molecule (eg.lagerhans cells and macrophages are APC involved in DTH response). A subsequent exposure to the SRBC antigen induces the effector phase of the DTH response, where T_{H1} cells secrete a variety of cytokines that recruits and activates macrophages and other non specific inflammatory mediators. The delay in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages.

Evaluation of immunomodulatory activity of H_2 antagonists on the parameters of non specific immunity was carried out by *in vivo* phagocytosis and survival from cecal ligation and puncture induced abdominal sepsis.

The reticuloendothelial system (R.E.S.) is composed in part of network of reticular (supporting or structural) cells of the spleen, thymus and other lymphoid tissues, together with cells lining the sinuses of the spleen, bone marrow, and lymph nodes and capillary endothelium of the liver (Kuffers cells), and of the adrenal and pituitary glands. These comprise the sessile or fixed macrophages. In addition, free macrophages, such as the blood monocytes and other leucocytes and the tissue macrophages, are transported by the body fluids or wander through the tissues. The R.E.S. is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense. Various dyes, carbon particles, or Thorotrast (thorium dioxide), injected intravenously into animals, carbon is removed by sessile intravascular phagocytes in the liver and spleen. The Kuffer cells of liver take up approximately 90% and the splenic macrophages 10%. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen, from the bloodstream is a measure of reticuloendothelial phagocytic activity [10]. The effectiveness of phagocytosis in removing microorganisms from the blood has often been demonstrated experimentally.

In carbon clearance test, Ranitidine (62.5 mg/kg) (i.p.) and Famotidine (80mg/kg, p.o.) treated groups, exhibited significantly high phagocytic index as compare to control group. This indicates stimulation of the reticuloendothelial system by H_2 antagonist's treatment. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by opsonisation of the parasite with the antibodies and complement activation leading to more rapid clearance of parasites from blood.

The above outcome is substantiated by observing effect of H_2 antagonists on Cecal Legations and Puncture induced abdominal peritonitis, which is associated with major cause of morbidity and mortality following trauma and abdominal surgery. A factor, which influences recovery from such an infective process, is the host defense mechanism. The percentage survival in Ranitidine (62.5 mg/kg) (i.p.) and Famotidine (80mg/kg, p.o.) treated groups, was 90% and 80% respectively as against 50% survival in the control group after 48hr of CLP. After 168hr of CLP percentage survival in Ranitidine (62.5 mg/kg) (i.p.) and Famotidine (80mg/kg, p.o.) was found to be 20% and 20% respectively as compared to 0% in control group. H_2 antagonists treated groups protect mice against Cecal Legations and Puncture induced abdominal peritonitis for a longer period of time i.e 7 days. This indicates that H_2 antagonists enhanced capacity of the monocyte macrophage system.

Table 1: Effect of some H_2-antagonists treatment on primary antibody titer

S. NO	GROUP	TREATMENT	DOSE AND ROUTE	ANTIBODY TITER
1	Control	Distilled water	1ml/Kg (p.o.)	5.40 ± 0.244
2	Cyp. Control	Cyclophosphamide (Cyp.)	100mg/Kg (p.o.)	3.20 ± 0.375**
3	RAN Treated	Ranitidine (RAN)	62.5 mg/Kg (i.p.)	6.80 ± 0.2*
4	FAM Treated	Famotidine (FAM)	80 mg/Kg (p.o.)	5.8 ± 0.374
6	RAN + Cyp. Treated	Ranitidine + Cyclophosphamide	RAN 62.5 mg/Kg (i.p.) + Cyp.100mg/Kg (p.o.)	5.40 ± 0.24***
7	FAM + Cyp. Treated	Famotidine + Cyclophosphamide	FAM80mg/Kg (p.o.)+ Cyp.100mg/kg (p.o.)	5.40 ± 0.24***

Values are expressed as Mean ± S.E.M.
* = p< 0.05, ** = p<0.01 and *** = p<0.001
Immunosuppressed drug Treated groups were compared with cyclophosphamide control.
Cyclophosphamide control compared with control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Table 2: Effect of some H2-antagonists treatment on secondary antibody tir

S. NO	GROUP	TREATMENT	DOSE AND ROUTE	ANTIBODY TITER
1	Control	Distilled water	1ml/Kg (p.o.)	7.40 ± 0.245
2	Cyp.Control	Cyclophosphamide (Cyp.)	100mg/Kg (p.o.)	5.60±0.245**
3	RAN Treated	Ranitidine (RAN)	62.5 mg/Kg (i.p.)	10.2 ± 0.374**
4	FAM Treated	Famotidine (FAM)	80 mg/Kg (p.o.)	9.40±0.245*
6	RAN + Cyp Treated	Ranitidine (RAN) + Cyclophosphamide (Cyp.)	RAN 62.5 mg/Kg (p.o.) + Cyp.100mg/Kg (p.o.)	6.60 ± 0.245**
7	FAM + Cyp Treated	Famotidine (FAM) + Cyclophosphamide (Cyp.)	FAM 80mg/Kg (p.o.) + Cyp.100mg/Kg (p.o.)	6.80 ± 0.374**

Values are expressed as Mean ± S.E.M.
* = p< 0.05, ** = p<0.01 and *** = p<0.001
Immunosuppressed drug Treated groups were compared with cyclophosphamide control.
Cyclophosphamide control compared with control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

Table 3: Effect of some H2-antagonists treatment on Reticulo-Endothelial System by Carbon Clearance Test

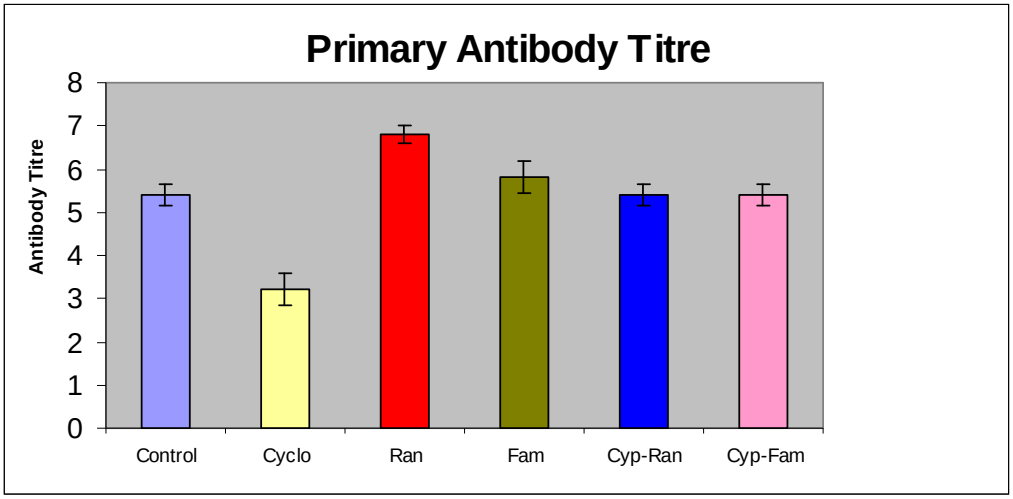
S. NO	GROUP	TREATMENT AND DOSE PER DAY.	PHAGOCYTIC INDEX
1	Control	Distilled water 1ml/Kg (p.o.)	0.0116 ± 0.00041
2	RAN Treated	Ranitidine 62.5 mg/Kg (i.p.)	0.0336 ± 0.00081***
3	FAM Treated	Famotidine 80 mg/Kg (p.o.)	0.0248 ± 0.00014***

Values are expressed as Mean ± S.E.M.
* = p< 0.05 and ** = p<0.01 and *** = p<0.001
Drug treated groups were compared with Control.
(Statistically analysed by one-way ANOVA followed by Turky-Kramar multiple comparisons test.)

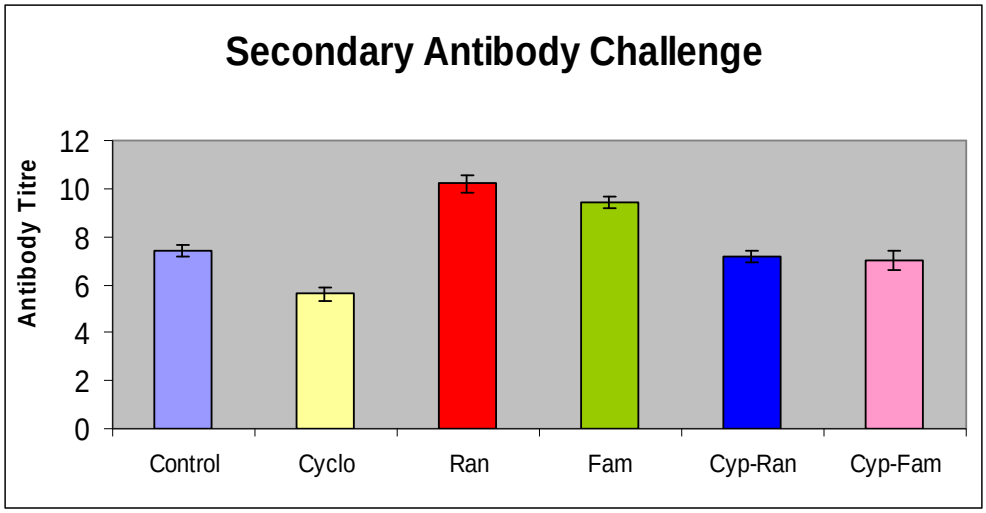
Table 4: Effect of some H2-antagonists treatment on Cecal Ligation and Puncture Induced abdominal peritonitis

S. NO	GROUP	TREATMENT	DOSE AND ROUTE	% SURVIVAL (12 HR.)	% SURVIVAL (24 HR.)	% SURVIVAL (48 HR.)	% SURVIVAL (168HR.)
1	Laparat Control	Distilled water	1ml/Kg (p.o.)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
2	CLP Control	Distilled water	1ml/Kg (p.o.)	60 ± 11.54	53.33 ± 6.6	6.6 ± 6.6	0 ± 0.0
3	RAN Treated (CLP)	Ranitidine	62.5 mg/kg (i.p.)	100 ± 0.0**	100 ± 0.0**	93.3 ± 6.6**	26.6 ± 6.6**
4	FAM Treated (CLP)	Famotidine	80 mg/Kg (p.o.)	100 ± 0.0**	86.6 ± 6.6**	80 ± 0.0**	26.6 ± 6.6**

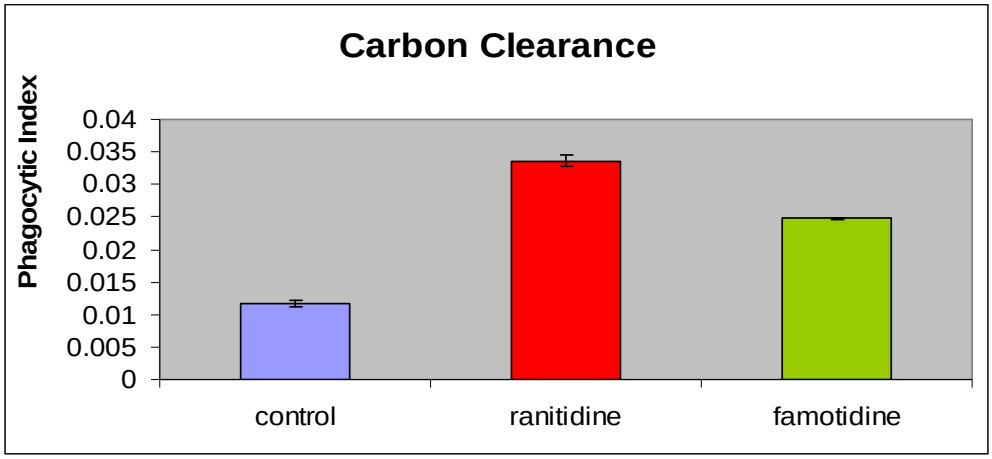
Values are expressed as Mean ± S.E.M.
* = p< 0.05 and ** = p<0.01 and *** = p<0.001
Drug treated groups were compared with Control.
(Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)



Graph 1: Effect of some H2-antagonists treatment on primary antibody titer. Immunosuppressed Drug Treated groups were compared with cyclophosphamide control. Cyclophosphamide control was compared with Control. (Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)



Graph 2: Effect of some H2-antagonists treatment on secondary antibody titer. Immunosuppressed Drug Treated groups were compared with cyclophosphamide control. Cyclophosphamide control was compared with Control. (Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)



Test Drug treated groups were compared with Control (Statistically analysed by one-way ANOVA followed by Tukey-Kramar multiple comparisons test.)

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