

Full Length Research Paper

Role of histamine H₂ receptor agonist and antagonist on liver function impairment in immunized rabbits

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This study was designed to explore the functional role of histamine H₂-receptors agonist and antagonist in the development of hepatic function impairment in immunized rabbits. The study comprised six groups containing 18 rabbits each. Groups III-VI received histamine (100 µg/kg, s.c.), H₂R-agonist (amthamine, 10 µg/kg, s.c.), H₂R-antagonist (ranitidine, 10 mg/kg, i.m.), and histamine (100 µg/kg, s.c.) plus H₂R-antagonist (ranitidine, 10 mg/kg, i.m.), respectively, b.i.d. for 10 days. Group I (negative control) and Group II (positive control) received sterile distilled water intramuscularly b.i.d. for 10 days. Groups II-VI were immunized on day 3 with intravenous injection of sheep red blood cells (1 × 10⁹ cells/ml). Blood samples were collected prior to immunization day 0, as well as on days 7, 14, 21, 28, and 58 post-immunization. Biochemical parameters ALT, AST, ALP, and bilirubin were determined. On each experimental day, the mean values of serum enzymes (AST, ALT, and ALP) and bilirubin (TB, DB, and IB) in negative and positive controls showed no significant changes while in Group III (histamine), IV (amthamine), V (ranitidine), and VI (ranitidine+histamine), these enzymes and bilirubin levels showed significant changes (p < 0.05), when compared with their values within group. The levels of serum enzymes and bilirubin showed significant difference (p < 0.05) in the group of histamine, amthamine, ranitidine, and ranitidine+histamine on each experimental day, when compared with the corresponding values of each other, and also compared with the corresponding values of negative and positive controls. Histamine, amthamine, ranitidine, and combination of ranitidine+histamine cause liver function impairment in terms of altered serum enzymes and bilirubin levels.

Key words: Histamine, HTMT, agonist, antagonist, ALT, AST, ALP, bilirubin, rabbit.

INTRODUCTION

Histamine is a mediator in processes related to allergy

and inflammation, or a neurotransmitter. Histamine exerts its effect through H₁, H₂, H₃, and H₄ receptors. In particular, the histamine H₂ receptor (H₂R) is coupled to the Gs protein/adenylyl cyclase system in a variety of tissues (e.g., brain, stomach, heart, gastric mucosa, and lung) (Kim et al., 2006; Shahid et al., 2009, 2010). Development of highly selective H₂R agonists (e.g., dimaprit, 4-(S)-methylhistamine, impromidine, and amthamine) and antagonists (e.g., ranitidine, tiotidine, burimamide, cimetidine, and famotidine) has greatly facilitated characterization of the multiple biological actions regulated by this receptor (Valle and Gantz, 1997). H₂R antagonists (e.g.

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Abbreviations: H₁R, H₁-receptors; H₂R, H₂-receptors; H₃R, H₃-receptors; H₄R, H₄-receptors; s.c., subcutaneous; i.m., intramuscular; b.i.d., bis in die; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin; TNF-α, tumor necrosis factor-alpha; PBS, phosphate buffered saline; i.v., intravenously; LFT, liver function tests.

ranitidine, cimetidine, and famotidine) have been developed and used clinically as antiacid secretagogues (Kim et al., 2006). Moreover, H₂R blockers are effective for the treatment of chronic heart failure (Kim et al., 2006). Dimaprit (a H₂R agonist) reduced the increase in plasma tumor necrosis factor- α (TNF- α) and L-alanine aminotransferase, while it reversed by cimetidine (a H₂R antagonist), and suggest that activation of the H₂R down-regulates the production of TNF- α and ALT (Nakamura et al., 1997). Fisher and Le Couteur (2001) suggested that renal and hepatic adverse effects resolved quickly after cessation of H₂R antagonist therapy and did not require specific treatment. Nephrotoxicity and hepatotoxicity following administration of a H₂R antagonist is rare and a high index of suspicion is necessary for early detection. Several H₂R antagonists are available over the counter, awareness of these conditions and early detection with cessation of H₂R antagonist therapy would appear paramount (Fisher and Le Couteur, 2001). Among three H₂R antagonist (cimetidine, ranitidine, and famotidine), cimetidine has the lowest toxicity and ranitidine has the highest toxicity, as judged from data on DNA synthesis and the total protein content of cultured hepatocytes, leakage of aminotransferases from the cells and morphological observations (Hirono et al., 1987). Moreover, H₂R antagonists, cimetidine and ranitidine have inhibitory effect on liver regeneration but famotidine has no such effect (Kanashima and Kobayashi, 1989). It has also been demonstrated that modest inflammation predisposes liver to ranitidine toxicity in rats and suggested a role for inflammation in idiosyncratic reactions to ranitidine (Luyendyk et al., 2003).

The purpose of this study was to test the hypothesis that underlying inflammation triggered by an antigen sheep red blood cells (SRBC) (nonhepatotoxic and non-immunotoxic) renders histamine, amthamine, ranitidine, and ranitidine plus histamine liver function impairment in rabbits.

MATERIALS AND METHODS

Drugs

Histamine dihydrochloride (Himedia Laboratories Pvt Limited, India), H₂R-agonist (amthamine dihydrobromide) (kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom); H₂R-antagonist (ranitidine hydrochloride) (Rantac[®], J. B. Chemicals and pharmaceuticals, India).

Dosing regime

Histamine dihydrochloride (100 μ g/kg) and H₂R-agonist (amthamine dihydrobromide) (10 μ g/kg) were administered through subcutaneous (s.c.) route, and H₂R-antagonist (ranitidine hydrochloride) (10 mg/kg) was administered through intramuscular (i.m.) route; twice in a day [12 h (8 am and 8 pm)] for 10 days (starting from 3 days prior to immunization until 7 days after immunization). All doses referred to the weight of the salts used.

Experimental design

To evaluate the serum level of liver enzymes and bilirubin, 108 (54 males and 54 females) New Zealand adult healthy albino rabbits of either sex weighing 1.0 - 1.5 kg were randomized equally into six treatment groups. Each group contained 18 (9 males and 9 females) rabbits. Group I (negative control) and Group II (positive control) received vehicle (sterile distilled water, 1 ml/kg \times b.i.d.) while Groups III, IV, V, and VI treated with histamine, H₂R-agonist, H₂R-antagonist and H₂R-antagonist plus histamine, respectively. They were housed in well maintained animal facility at Central Animal House, J. N. Medical College and Hospital, Aligarh Muslim University, Aligarh. This animal house was located in the Bioresources unit under a 12 h light/dark cycle and a temperature of 22 \pm 2°C and the animals were allowed free access to standard laboratory diet including green vegetables and tap water during the studies. All studies were carried out during the light cycle and were approved by the Institutional Animal Ethical Committee at J. N. Medical College and Hospital.

Antigen

Sheep blood diluted 1:1 in sterile Alsevier's solution was obtained from Department of Microbiology, J. N. Medical College and Hospital, A.M.U., Aligarh, and washed with phosphate buffered saline (PBS) (pH 7.4) thrice by centrifugation. The cell suspensions were adjusted to the desired concentration in terms of hemoglobin as previously described (Tripathi et al., 2010a-f). Briefly, lysis of a 1% SRBC suspension (2×10^8 cells/ml) with 14 volumes of 0.1% Na₂CO₃ develops an optical density of 0.135 at 541 nm in a spectrophotometer. Finally, the concentration was adjusted to 5% (1×10^9 cells/ml) in PBS for immunization before use.

Immunization of rabbits

All the rabbits in the Groups (II - VI) were immunized on day 3 intravenously (i.v.) with 1 ml of 5% (1×10^9 cells/ml) SRBC in PBS.

Biochemical analyses

To determine biochemical levels for liver functions, 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°C overnight. Blood samples were centrifuged for 10 min at 580 \times g, and serum was separated and heated at 56°C for 30 min to inactivate complement proteins and stored in aliquots containing sodium azide as preservative at -20°C. Serum levels of liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)] and bilirubin [total bilirubin (TB), direct bilirubin (DB), and indirect bilirubin (ID)] were determined using an automatic analyzer (Transasia XL 300, Germany).

Statistical analyses

Data were summarized as Mean \pm SD. Groups were compared by using repeated measures (subjects within groups) two way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. A two-tailed ($\alpha = 2$) probability $p < 0.05$ was considered to be

RESULTS

The changes in the mean values of serum level of serum

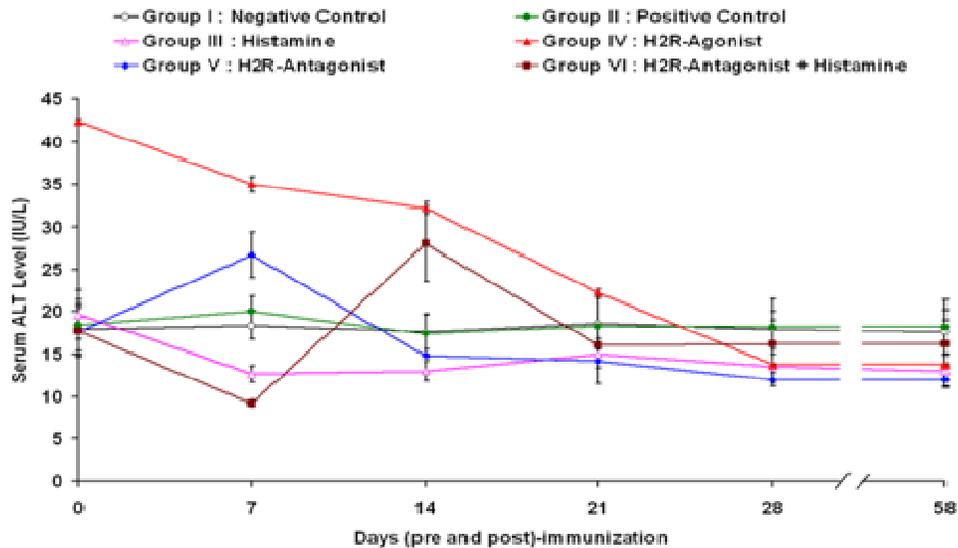


Figure 1. Effects of histamine and H₂-receptors agonist and antagonist on serum ALT levels. The results demonstrate mean ± SD. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F = 1163.937, DF = 5,102; p < 0.01) and days (F = 237.363, DF = 5,510; p < 0.01) on SRBC were statistically significant. The interaction (treatments × days) effect of (F = 285.644, DF = 25,510; p < 0.01) these on SRBC were also found to be significant.

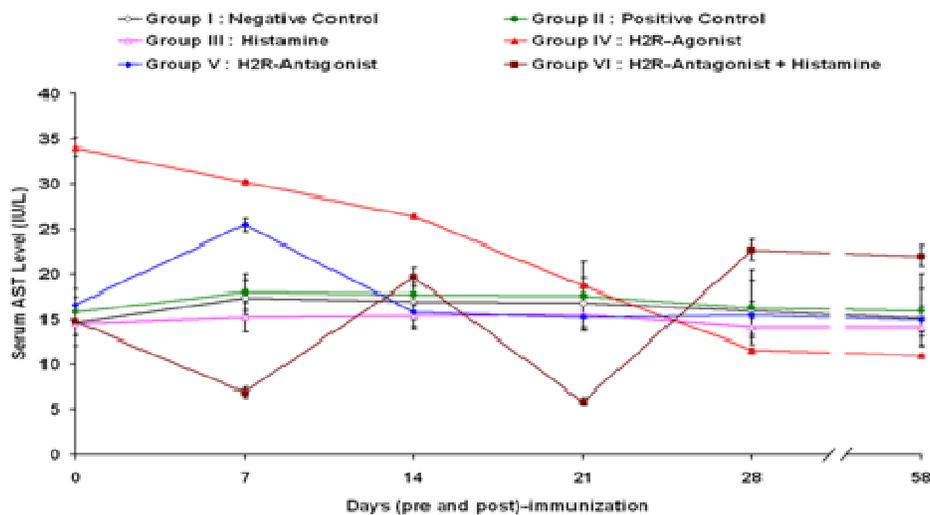


Figure 2. Effects of histamine and H₂-receptors agonist and antagonist on serum AST levels. The results demonstrate mean ± SD. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F = 482.721, DF = 5,102; p < 0.01) and days (F = 122.566, DF = 5,510; p < 0.01) on SRBC were statistically significant. The interaction (treatments × days) effect of (F = 177.821, DF = 25,510; p < 0.01) these on SRBC were also found to be significant.

enzymes (ALT, AST, and ALP) and bilirubin (TB, DB, and IB) in Groups I – VI are shown in Figures 1, 2, 3, 4, 5, and 6, respectively.

On each experimental day, the mean values of serum level of serum enzymes (ALT, AST, and ALP) and

bilirubin (TB, DB, and IB) in Group I (negative control) and Group II (positive control) showed no significant difference, when compared with values of serum enzymes (ALT, AST, and ALP) and bilirubin (TB, DB, and IB) within group, and also compared with the corresponding values

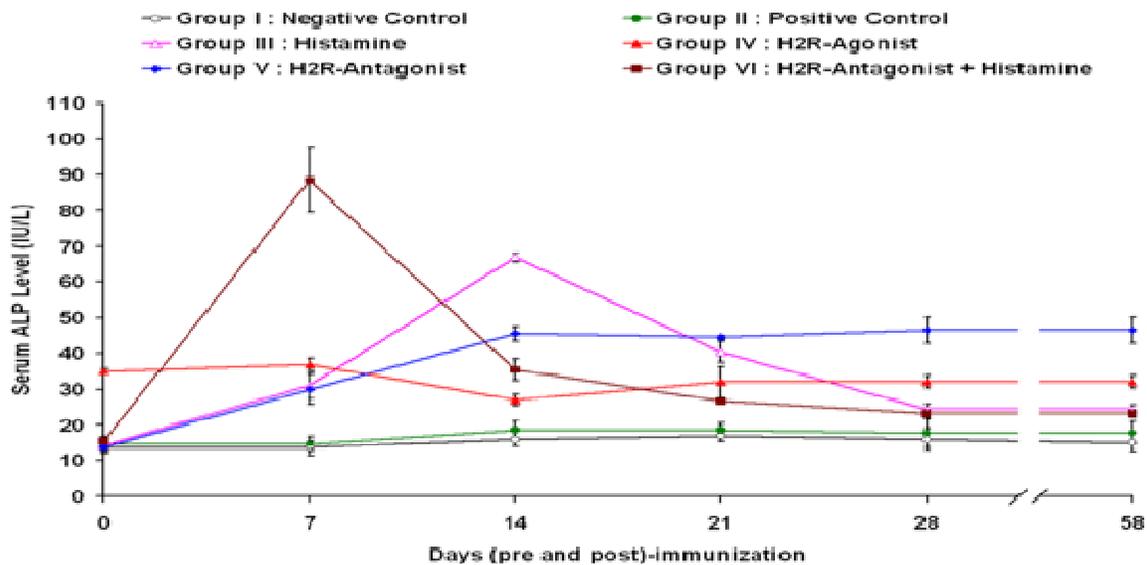


Figure 3. Effects of histamine and H₂-receptors agonist and antagonist on serum alkaline phosphatase (ALP) levels. The results demonstrate mean \pm SD. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F = 696.678$, $DF = 5,102$; $p < 0.01$) and days ($F = 742.821$, $DF = 5,510$; $p < 0.01$) on SRBC were statistically significant. The interaction (treatments \times days) effect of ($F = 276.182$, $DF = 25,510$; $p < 0.01$) these on SRBC were also found to be significant.

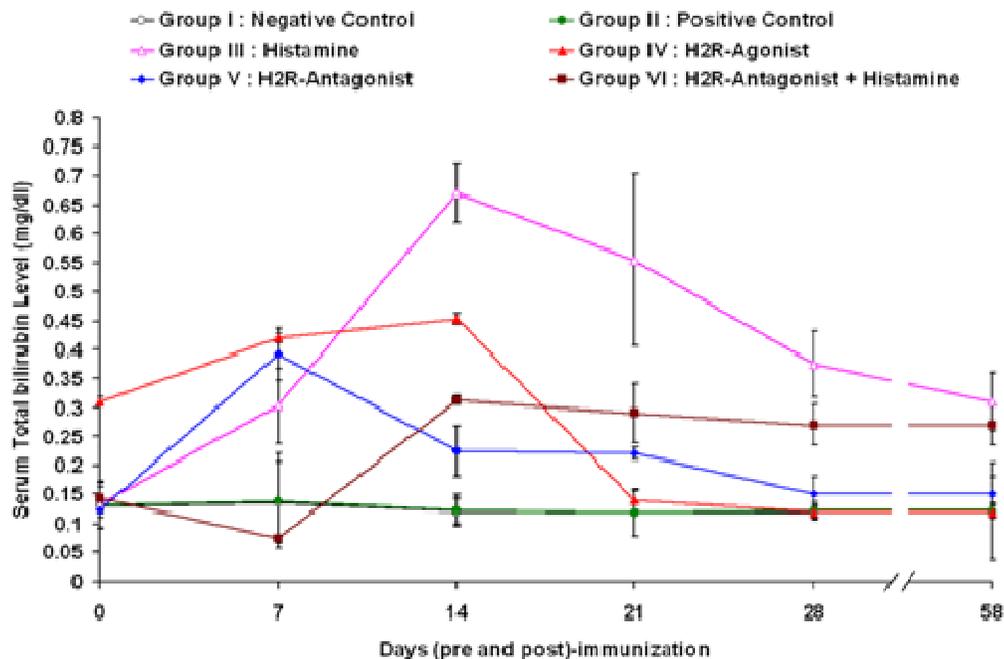


Figure 4. Effects of histamine and H₂-receptors agonist/antagonist on serum total bilirubin (TB) levels. The results demonstrate mean \pm SD. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments ($F = 775.278$, $DF = 5,102$; $p < 0.01$) and days ($F = 199.418$, $DF = 5,510$; $p < 0.01$) on SRBC were statistically significant. The interaction (treatments \times days) effect of ($F = 134.927$, $DF = 25,510$; $p < 0.01$) these on SRBC were also found to be significant.

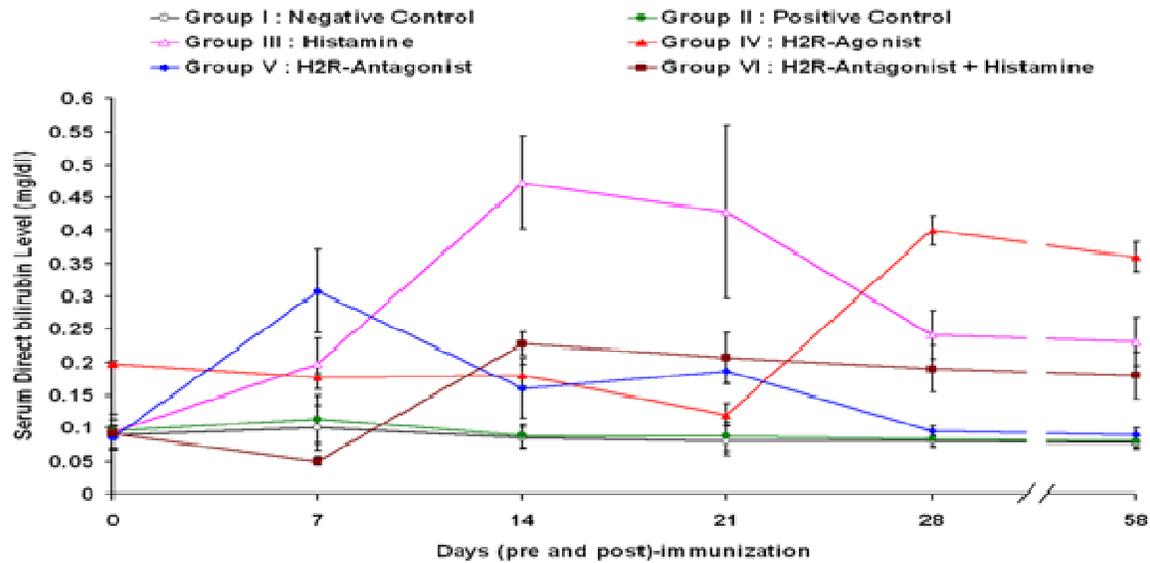


Figure 5. Effects of histamine and H₂-receptors agonist and antagonist on serum direct bilirubin (DB) levels. The results demonstrate mean ± SD. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F = 469.121, DF = 5,102; p < 0.01) and days (F = 142.24, DF = 5,510; p < 0.01) on SRBC were statistically significant. The interaction (treatments × days) effect of (F = 111.402, DF = 25,510; p < 0.01) these on SRBC were also found to be significant.

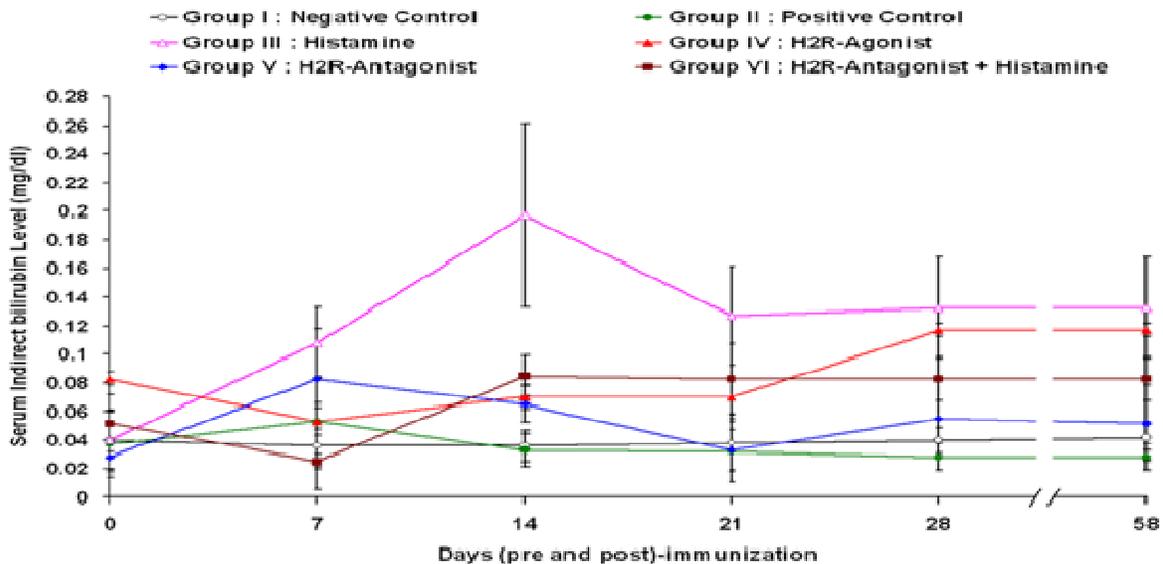


Figure 6. Effects of histamine and H₂-receptors agonist and antagonist serum indirect bilirubin (IB) levels. The results demonstrate mean ± SD. of three experiments each with six rabbits. Two-way ANOVA followed by Newman-Keuls post hoc test revealed that the effect of treatments (F = 71.109, DF = 5,102; p < 0.01) and days (F = 6.737, DF = 5,510; p < 0.01) on SRBC were statistically significant. The interaction (treatments × days) effect of (F = 7.876, DF = 25,510; p < 0.01) these on SRBC were also found to be significant.

corresponding values of each other. In Groups III (histamine), IV (amthamine), V (ranitidine), and VI (ranitidine plus histamine), these enzymes (ALT, AST, and ALP) and bilirubin (TB, DB, and IB) levels showed significant difference (p < 0.05) on each experimental day, when

compared with values of serum enzymes and bilirubin within group.

Furthermore, the levels of serum enzymes (ALT, AST, and ALP) and bilirubin (TB, DB, and IB) showed significant difference (p < 0.05) in the group of histamine,

amthamine, ranitidine, and ranitidine plus histamine on each experimental day, when compared with the corresponding values of each other, and also compared with the corresponding values of negative control and positive control.

DISCUSSION

Imoto et al. (1985) have quantitatively determined the presence of histamine receptors (H_1R s and H_2R s) on liver plasma membrane. They showed that the numbers of the H_1R s were greatest (4740 ± 750 fmol/mg protein) than the numbers of the H_2R s (116 ± 11 fmol/mg protein). Recently, toxicological study demonstrated that liver of rabbits was markedly damaged by histamine and histamine receptors agonists (Tripathi et al., 2010g). These observations revealed that histamine and H_2R -agonist caused to hepatic congestion while centrilobular necrosis is caused by H_1R -agonist. H_2R -agonist also reveals unusual multinuclearity, when compared with control rabbits. Recently, our laboratory has demonstrated the roles of histamine receptors (H_1R , H_2R , H_3R , and H_4R) in immune modulation and regulation by using histamine receptors agonists and antagonists in SRBC immunized and non-immunized rabbits (Tripathi et al., 2010a-f). Notably that the SRBC assay does not describe the mechanism of immunosuppression. Also, SRBC is used as the particular T-lymphocyte dependent antigen and demonstrates the coordinated interaction of various immune system cells (Kognai et al., 2007). According to the document of the "International Conference on Harmonization (ICH) S8 Guideline on Immunotoxicity Testing for Pharmaceuticals", the evaluation of antibody response to a T-lymphocyte dependent antigen (SRBC or Keyhole limpet hemocyanin) is recommended as one of the most sensitive immune tests following chemical exposures (Kognai et al., 2007).

In addition to the further study of SRBC toxicity followed by chemical exposures, we have designed our study to explore histamine and H_2R -agonist and antagonist induced liver function impairment in SRBC immunized and non-immunized rabbits over a period of 58 days.

Liver is the most important organ, which plays the central role in regulating various physiological processes in the body. It is involved in several imperative functions including metabolism, secretion, and storage. It has immense capacity to detoxicate toxic substances and synthesize useful principles. Hence, damage to the liver inflicted by hepatotoxic agents is of serious consequences. Liver diseases are mainly caused by toxic chemicals, drugs, excessive consumption of alcohol, infections, and autoimmune disorders (Gutiérrez and Solís, 2009). The abnormalities in liver function tests (LFTs) are elevated levels of static biochemical tests [e.g., liver enzymes (ALT, AST, and alkaline phosphatase) and bilirubin], which are biochemical parameters of liver

injury (Masaki et al., 2005; Thapa and Walia, 2007). The biotransformation of xenobiotics or drugs takes place in liver (Maciejewska-Paszek et al., 2007); therefore, the LFTs are the most common blood tests required by clinical practitioners for examining liver function impairment. The most common abnormalities of LFTs in an asymptomatic person are augmented AST and ALT (Giannini et al., 2005). However, serum alkaline phosphatase (ALP) activity may be derived from liver, bone, intestine, and placenta (Hasan and Owyed, 2003). The results of this study revealed that Groups III-VI show impaired serum levels of ALT, AST, and ALP. Group IV (amthamine), Group V (ranitidine), and Group VI (ranitidine plus histamine) show moderate alterations in serum levels of ALT and AST while Group III (histamine) shows mild alterations in serum levels of ALT and AST, when compared with Group II (positive control) and Group I (negative control). However, all drug treated Groups (III - VI) show moderate alterations in serum level of ALP, when compared with Groups I and II.

Furthermore, serum bilirubin is a mixture of α , β , γ , and δ fragments which are unconjugated, singly conjugated, doubly conjugated, and covalently bound to albumin, respectively (Yap and Aw, 2010). In most cases a total bilirubin (TB) assay suffices for LFT, but fractionation may be required in isolated increases in bilirubin and neonatal jaundice. Direct bilirubin (DB) refers to the conjugated bilirubins that react directly with the diazo reagent, while indirect bilirubin (IB) is a derived value obtained from the difference of the TB and the DB. The DB assays measure only 70 - 90% of the conjugated and δ bilirubins, and may underestimate the severity of jaundice (Yap and Aw, 2010). Serum bilirubin is useful in separating the causes of jaundice. In prehepatic jaundice due to haemolysis, unconjugated bilirubin is increased with little or no increase in conjugated bilirubin. In hepatic and post-hepatic jaundice, there is increased conjugated and δ bilirubin (Yap and Aw, 2010). When the liver functions tests are abnormal and the serum bilirubin levels are more than $17 \mu\text{mol/L}$, they suggest underlying liver disease (Friedman et al., 2003, Thapa and Walia, 2007). The bilirubin results of this study revealed that the serum levels of TB, DB, and IB in Groups III-VI show moderate alterations in liver function impairment, when compared with positive control and negative control.

In this study, we have observed that the serum levels of enzymes (ALT, AST, and ALP) and bilirubin levels (TB, DB, and IB) in positive control (SRBC-immunized) and negative control (non-immunized) show similar patterns. Hence, the alterations in serum enzymes and bilirubin levels in Groups (III - VI) were due to receptor based ligands treatment.

Conclusion

It is therefore concluded that histamine, amthamine (H_2R -agonist), ranitidine (H_2R -antagonist), and combination of

ranitidine plus histamine cause hepatic function impairment in terms of altered serum enzymes and bilirubin levels. Thus, the SRBC is non-hepatotoxic and a suitable antigen for immunotoxic studies. To the best of our knowledge, this is the first report describing comparative study of histamine and H₂R-agonist and antagonist on liver function in immunized and non-immunized rabbits.

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