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EFFECT OF COLCHICINE ON HUMORAL IMMUNITY AND IMMUNE ORGANS IN MICE

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ABSTRACT

This study was planned to investigate effect of colchicine on humoral immune responses and immune organs in mice. Humoral immune responses were evaluated by performing haemagglutination assay, mice lethality test and Jerne hemolytic plaque formation. Colchicine treated groups were administered 40µg/kg, 80µg/kg and 160µg/kgData obtained was analyzed by ANOVA. There was significant($P < 0.0001$)reduction in HA titer of colchicine treatedgroups. With the increasing dose, there was reduction in the HA titer. Colchicine enhanced the mortality after administration of bovine *Pasteurellamultocida*culture. In addition, there was significant ($P<0.0001$) reduction in body weight and spleen weight difference of mice in colchicine treated groups. There were no histopathological changes observed in spleen and thymus at 40µg/kg and 80µg/kg doses of colchicine. At 160µg/kg dose, increase in thickness of trabecular was seen due to edema in the spleen. This study has shown that humoral immunity is suppressed by the administration of colchicine.

Key words: Colchicine, Humoral immunity, Immune organs.

INTRODUCTION

Colchicine has been used for the treatment of biliary, liver cirrhosis, idiopathic thrombocytopenic pupura resistant to regular treatment and pain of gouty arthritis (Strother *et al*. 1984; Sabouraud *et al*. 1992; Lidar and Livneh 2007). Colchicine ointment is useful for treatment and prevention of acute gout attacks (Maduri and Atla 2012). Colchicine administration has blocked CappD-crystal and urate crystal activated inflammation. Inflammation occurs due to prostaglandin E1 (PGE1).This shows that colchicine plays its role as anti-PG agent (Denko 1975). Colchicine stopped increased movement of Polymorphonuclear cells in Behcet's disease patient (Jorizzo *et al*. 1984). Enhanced lymphocyte proliferation was induced by colchicine **(**Stenzel*et al*. 1978).

Plasma cells are responsible for production of B cell. They have ability to synthesize and secrete large number of antibodies of same type. B cells respond to particular microbes with specificity and movement of antibodies. Differentiation of B cells in to plasma cells was also studied (Miriam and Kathryn 2005). Humoral immunodeficiency could be investigated by measurement of immunoglobulin (Sandhya *et al*. 2010).Humoral or antibody immune responses are important for protection against bacteria. Lung has capability to react against pathogens by stimulation of memory B cells. IgA and IgG are produced having ability to destroy pathogens by acting systemically and locally (Twigg 2005).The current study was designed to evaluate effect of colchicine on humoral immunity and immune organs in mice.

MATERIALS AND METHODS

Effects of Colchicine on Humoral immunity

Experimental Animals: Five-to-seven week-old Albino mice were purchased from department of Theriogenolgy University of Veterinary and Animal Sciences (UVAS) Lahore and kept in animal house of the UVAS, Lahore, by taking into consideration all feasible sanitary measures. The animals were kept on standard pelleted diet and water *ad libitum*. All experimental manipulations were undertaken in agreement with the Institutional Guidelines for the Care and Use of Laboratory Animals Letter No. DR / 643 dated 22-06-2015.

Antigen: Sheep red blood cells (SRBCs) were obtained from department of microbiology UVAS, Lahore. *Pasteurellamultocida* culture was obtained from university diagnostic Laboratory,UVAS Lahore.

Chemicals: Colchicine (Applichempvt Ltd Germany), Cyclosporine (Santa Cruz Biotechnology, USA)

Experimental design: In each experiment, mice were divided into a group I (negative control), groups II (40µg/kg), group III (80µg/kg), group IV (160µg/kg) colchicine treated and group V (positive control).All these groups were administered doses intraperitoneally. Positive control group received cyclosporine 100µg/kg. Negative control group received phosphate buffer saline (PBS) only. Effect of colchicine was studied on humoral immunity and immune organs.Effect of colchicine on humoral immunity was studied by performing mice lethality test, haemagglutination assay and Jerne hemolytic plaque formation assay.

Haemagglutination Assay (HA): Colchicine treated and control groups were administered doses daily intraperitoneally for 28 days. Every mouse was immunized with 0.5×10^8 sheep red blood cells (SRBCs) intraperitoneally at 14th and 21st day of experiment, including positive and negative control groups. Blood was collected from each mouse at 28thday.Serum was separated from blood. Antibody titers against the SRBCs were determined in all treated groups by haemagglutination assay as described (Hassan *et al*. 2004). The maximum number of serum dilution expressing haemagglutination was shown as HA titer. HA titer value of colchicine treated groups was compared with the negative control (Fulzele *et al*. 2003).

Mice Lethality Test: Mice in colchicine treated groups were administered with 40µg/kg, 80µg/kg and 160µg /kg doses. Mice of negative control were treated with PBS and positive control group was treated cyclosporine 100µg/kg. All these above groups were treated for 21 days. The animals were immunized with haemorrhagicsepticaemic vaccine (HS vaccine) on 7th and 17th day of the treatment. Mice were challenged subcutaneously with 0.2 ml (25 lethal $dose_{50}$) of the *Pasteurellamultocida* culture containing 10^7 cells per mlon 21st day. The animals were observed for a period of 72 hours to detect any mortality in the inoculated mice. The mortality ratios were obtained as described earlier (Sudha*et al*. 2010).

$$\text{Mortality ratio} = \frac{\text{No. of animals dead}}{\text{Total no of animals}}$$

Jerne hemolytic plaque formation assay: This experiment was performed for finding antibody formation against SRBC as described previously (Jerne *et al*. 1974). Colchicine treated and control groups were administered doses for one week daily intraperitoneally. On 2nd day of the test, mice of colchicine treated and control groups were sensitized with 5% washed SRBCs.Each mouse was administered 0.2 ml of SRBCs. Four days after sensitization (6th day of the test), all mice were scarified and their spleens were separated , crushed and strained to form a suspension of single cells in 10 ml RPMI-1640 medium to prevent premature discharge of antibodies from the cells. The quantity of splenic cells defined was 1×10^6/mL of RPMI. This triturate was strained and 500 µL of filtrate was mixed with equivalent volume of 0.7% warm (45°C) agarose (Sigma Co, USA) in RPMI (2000 µL) and 2% SRBC.A uniform layer of this mixture was formed on autoclaved glass slides after pouring. It was incubated at 37°C for 2 h. After incubation, guinea pig complement10% in sterilize normal saline was mixed and an overnight incubation at 37°C was given for lysis of SRBC creating a clear circular plaque around every antibody producing cell (plasma cells). The plaques werecounted and recorded as the number of plaque forming cells (PFCs) per million cells (Sajid*et al*. 2007).

Effect on Immune organs: Colchicine treated and control groups were administered doses intraperitoneally daily for 28 days. After 28 days, mice were sacrificed by procedure of cervical dislocation. The thymus and spleen of mice were separated and weighed. At 28 days, histopathology was performed for the thymus and spleen of colchicine treated groups and control groups. A 5µm section of thymus and spleen were made for each group with help of section cutting machine. This section was treated with hemotyxin dye. Then, this was observed under microscope for any change in the organ histopathology (Hassan *et al*. 2004).

Statistical Analysis: The data was analyzed by one way analysis of variance (ANOVA) followed by multiple comparison test i.e. LSD (Least significant difference) with the help of statistical package of social sciences (SPSS). The values were considered significant at $P<0.05$.

RESULTS

Haemagglutination assay: The effect of colchicine on HA titer was shown in Figure 1.Colchicine treated groups presented a significant ($P<0.0001$) decrease in serum anti SRBC titer as compared to negative control. Colchicine at the dose of 160µg/kg showed lowest HA titer with significant ($P< 0.0001$) reduction from negative control. HA titer was more significantly decreased in 160µg/kg as compared to 40µg/kg and 80µg/kg treated groups. Although, there was significant reduction in HA titer at 40µg/kg and 80µg/kg treated groups of colchicine from negative control but this difference is less significant as compared to 160 µg/kg dose of colchicine. There was significant ($P<0.0001$) reduction in HA titer in positive control (cyclosporine 100µg/kg) as shown in Figure 1.

Mice lethality assay: This assay was performed to assess the influence of colchicine on humoral immunity. There was death of all the mice in the non-vaccinated group within 24 hour period. There was no death of mice in the negative control group in which there was administration of PBS and vaccine. At 40µg/kg dose of colchicine, there was 50% mortality. There was 75% mortality observed at 80µg/kg dose of colchicine. Maximum mortality was observed at the 160µg/kg colchicine dose i.e. 100%. There was 100% mortality observed in positive control (cyclosporine100µg/kg) as shown in Table 1.

Jerne hemolytic plaque formation assay: Thisassay was performed to evaluate production of antibodies producing plasma cells in the splenocytes in mice against SRBCs. The results were found significant in all groups of colchicine. Splenocytes obtained from colchicine groups II, III, IV and control groups were treatedagainst SRBCs. These splenocytes has antibodies. When SRBCs (antigen) were treated with antibodies, formation of plaques

indicated antigen antibody reaction. Dose dependent decrease in antibody producing plasma cell was observed in colchicine treatment groups as compared to negative control group. There was significant (P<0.0001) decrease in number of plaques from negative control to all doses of colchicine 40µg/kg, 80µg/kg and 160µg/kg. Antibody formation was decreased with increasing dose of colchicine. There was significant (P<0.0001) reduction in positive control group compared to negative control as shown in Figure 2.

Effect on Immune Organs: Colchicine was administered for 28 days to investigate its effect on immune organs such as spleen and thymus. Effect on these organs, body weight and histopathological analysis were also performed. Mice were weighed before start of experiment**.** There was significant (P<0.0001)decrease in difference of body weight of mice in colchicine group II (40µg/kg), group III (80µg/kg) and group IV (160µg/kg) to negative control after treatment. There was significant (P<0.0001) reduction in difference of body weight in positive control as compared negative control. Significant (P<0.0001) reduction in spleen weight was observed with increasing dose of colchicine in group II, group III and group IV. There was significant (P<0.0001) decrease in spleen weight after administration of positive control (cyclosporine). There was difference of weight of thymus in colchicine treated group II (40 µg/kg), group III (80µg/kg) and group IV (160µg/kg) to negative control but difference was statistically not significant. There was no significant difference of weight of thymus after administration of positive control (cyclosporine) as shown in Table 2.

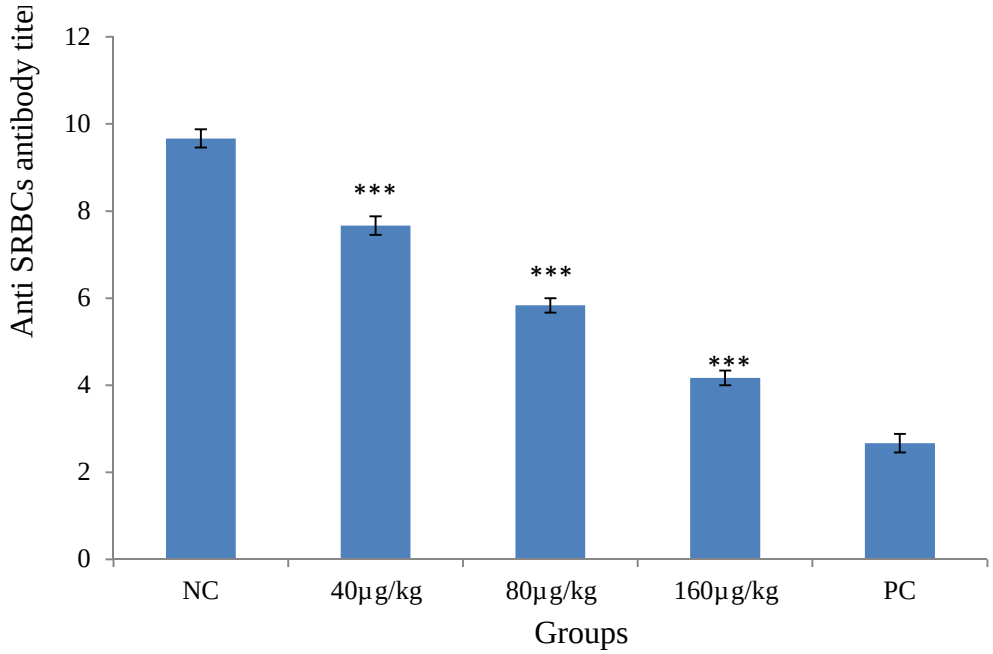

Figure 1. Log 2 value of antibody titer, ±SEM (n=6) *** p<0.0001 compared to negative control by using one way ANOVA .NC: negative control, PC: positive control

Table 1. Percentage mortality ratio of controls and colchicine treated groups.

Mortality at different time intervals	NegativeControl		Treatment groups			
	Vaccinated+PBS (n=8)	Non-vaccinated (n=8)	40µg/kg (n=8)	80µg/kg (n=8)	160µg/kg (n=8)	Positive control (n=8)
Mortality after 24 h	0	8	2	4	6	7
Mortality after 48 h	0	0	1	2	2	1
Mortality after 72 h	0	0	1	0	0	0
Mortality percentage	0	100%	50%	75%	100%	100%

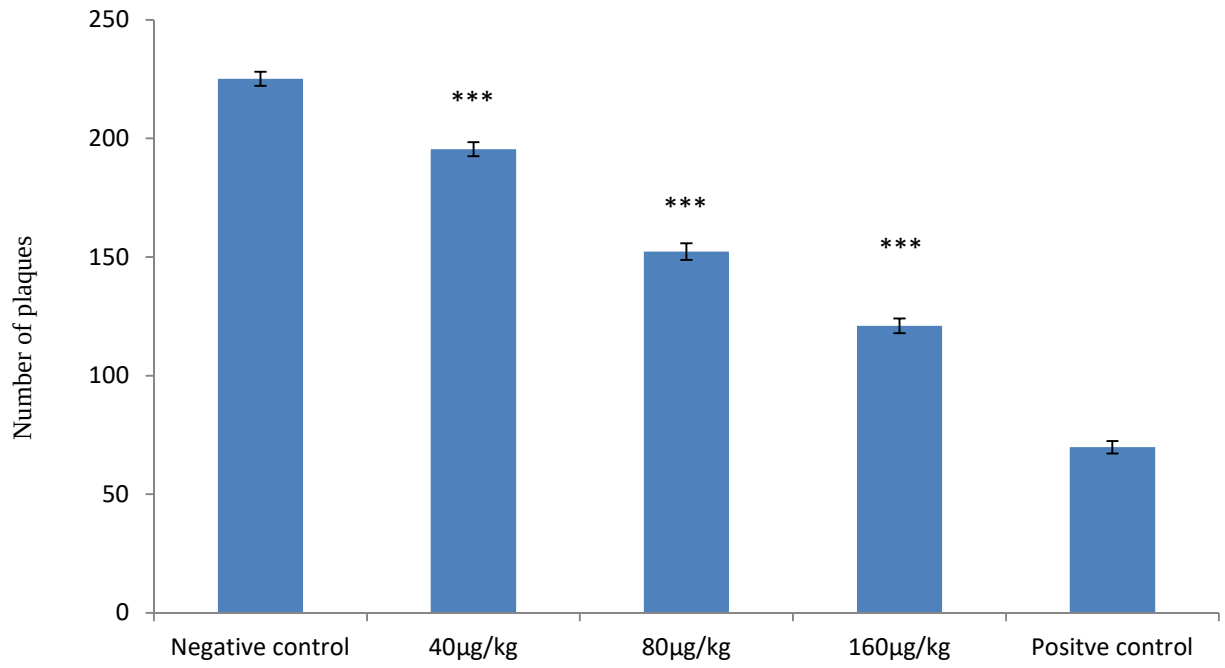

Figure 2.Number of plaques formed in colchicine 40 µg/kg, 80µg/kg, 160µg/kg ±SEM (n=6) ***p<0.0001 compared to negative control by using one way ANOVA, Data are represented as ±SEM

Table 2.Effect of colchicine on organ weight after daily IP injection .

Parameter	Negative control	40«g/kg	80«g/kg	160«g/kg	Positive control
Body weight before treatment (g)	19.98±0.390	22.8±0.464	26.96±0.398	28.42±0.755	32.1±0.357
Body weight after treatment (g)	31.8±0.759	29.8±0.547	29.14±0.781	30.04±0.698	33.78±0.143
Spleen weight(g)	0.322±0.0070	0.26***±0.005	0.214***±0.005	0.184***±0.0070	0.15±0.0086
Spleen weight (% of BW)	1.07±0.0167	0.87±0.015	0.68±0.0079	0.57±0.0146	0.47±0.0199
Thymus weight (g)	0.03±0.0009	0.024±0.0007	0.018±0.0007	0.013±0.0007	0.062±0.0139
Thymus weight (% of BW)	0.093±0.0014	0.081±0.0029	0.062±0.0033	0.043±0.0017	0.18±0.0371

Data are as mean ±S.E (n=5) ***P>0.0001 from negative control

Histopathological Assay: Mice were administered colchicine 40µg/kg, 80µg/kg and 160µg/kg daily for 28 days. Thymus and spleen were separated from each mouse and histopathological changes were observed. Thymus and spleen of mice of negative group has no significant histopathological changes. There was increase in thickness of trabecular at 160µg/kg dose. Readily detectable changes were observed in red pulp and white pulp ratio at 160µg/kg as shown in Figure 3. There was also presence of edema in the spleen at dose of 160µg/kg as shown in Figure 4.Few histopathological changes in thymus were observed. There were no histopathological changes observed in thymus of mice at 40µg/kg and 80µg/kg doses of colchicine. Mild atrophy and capsular changes were observed at 160µg/kg dose as shown in Figure 5.Histopathological changes were observed in thymus in positive control (cyclosporine).Capsular and trabecular changes were observed in positive control as shown in figure 6.Medulary atrophy was also observed. Spleen was observed after administration of positive control (cyclosporine) with decreased cellularity and changes in trabecular and capsular. There were no significant histopathological changes observed at doses 40µg/kg and 80µg/kg doses of colchicine in thymus and spleen of mice. Few changes in capsular trabecular were observed at 80µg/kg dose as shown in Table 3.

Table 3.Histopathological changes observed in thymus and spleen administered with different doses of Colchicine.

	Spleen				Thymus		
Treatment	**White pulpy Atrophy**	**Capsular Trabecular Changes**	**Odema in Spleen**	**Red pulp/white pulp ratio**	**Medularyatrophy**	**Starry sky atrophy**	**Capsular and trabecular Change**
Negative control	–	–	–	–	–	–	–
40µg/kg	–	–	–	–	–	–	–
80 µg/kg	–	+	–	–	–	–	–
160 µg/kg	–	–	++	++	++	–	++
Positive Control	–	–	-	++	++	–	++

–:No changes observed; +:minimal changes; ++: readily detectable changes; +++: vigorous changes detected

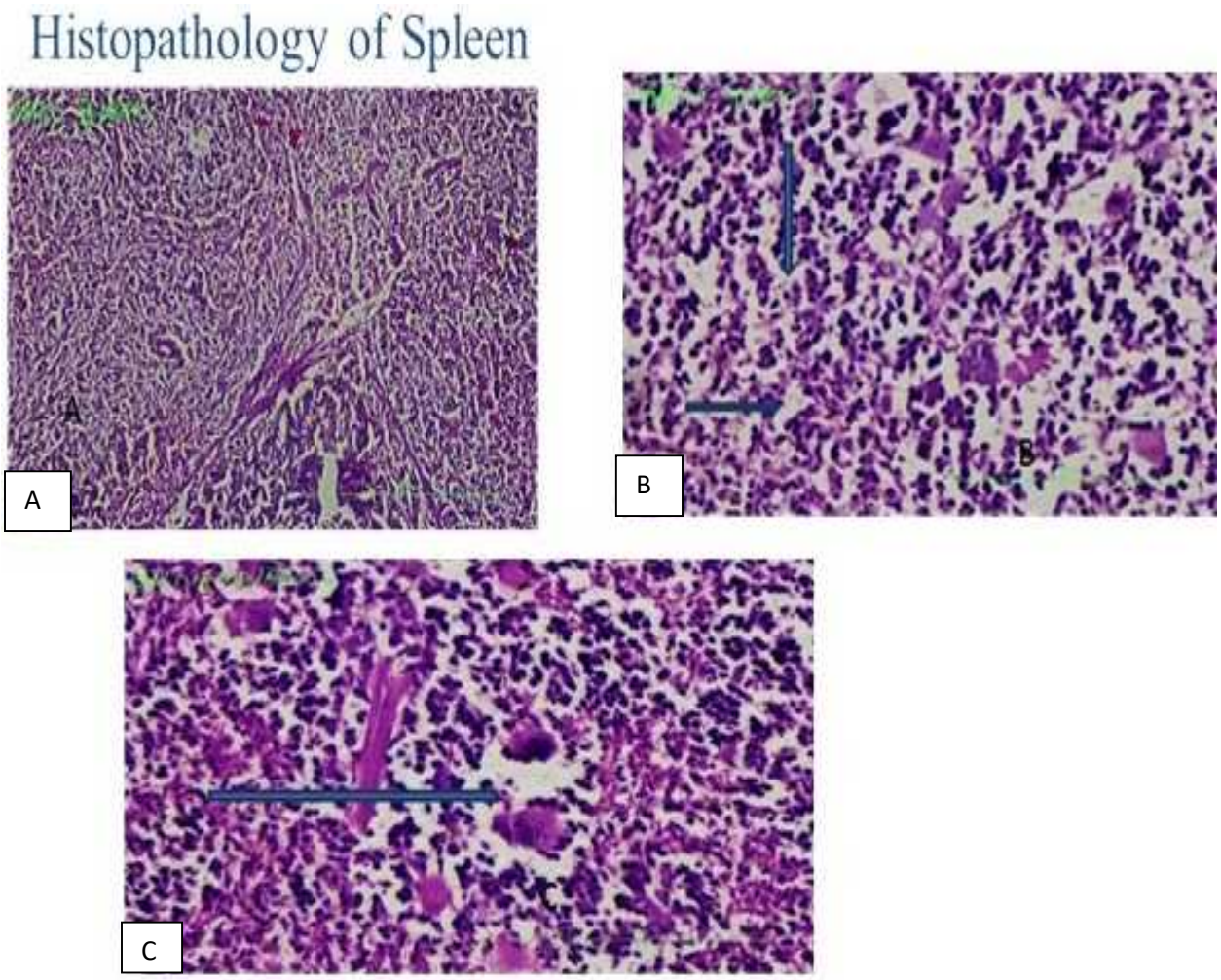

Figure 3 Histological analysis of spleen of mice after administration of colchicine
(A) Showing normal spleen cell arrangements with no change (B) & (C) showing decreased cellularity, trabecular and capsular changes seen after Colchicine administration.

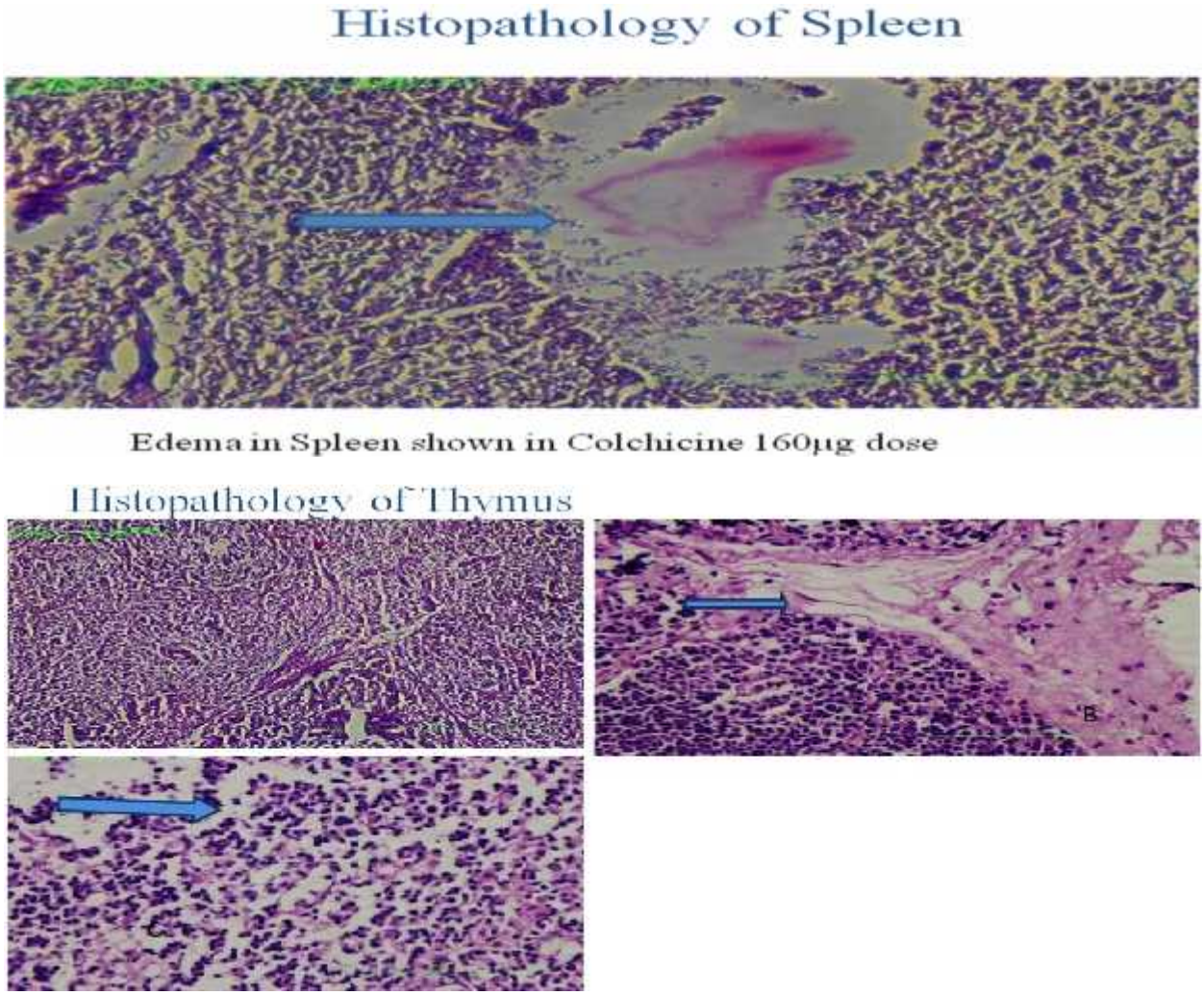

Figure 5.Histological analysis of Thymus of mice after Colchicine administration viewed at X-40. (A) Showing normal thymus cell with no changes seen in control group (B &(C) indicating medullary atrophy, Capaular& trabecular change after 160 «g/kg administration of colchicine.

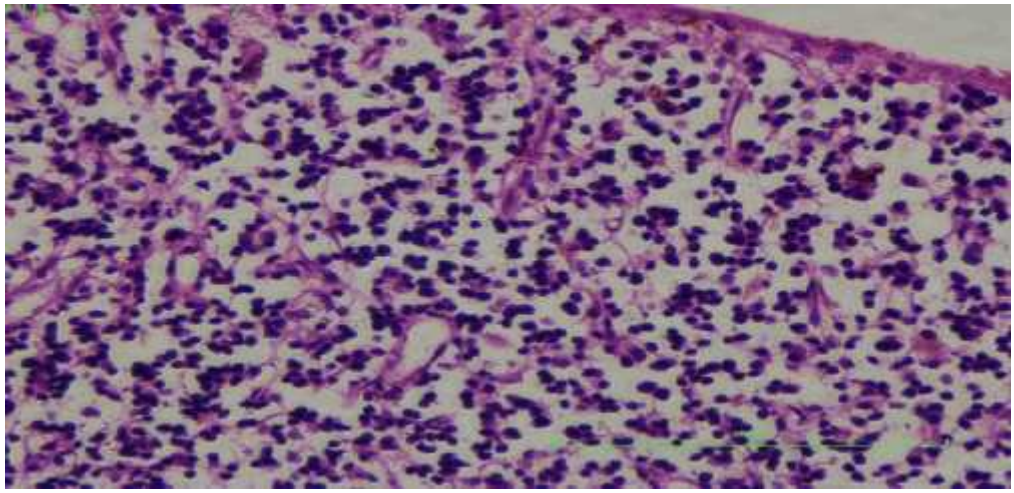

Figure 6 Histopathological analysis of thymus of mice after Positive control (Cyclosporine) 100«/kg administration viewed at 400X indicating Capsular and trabecular changes.

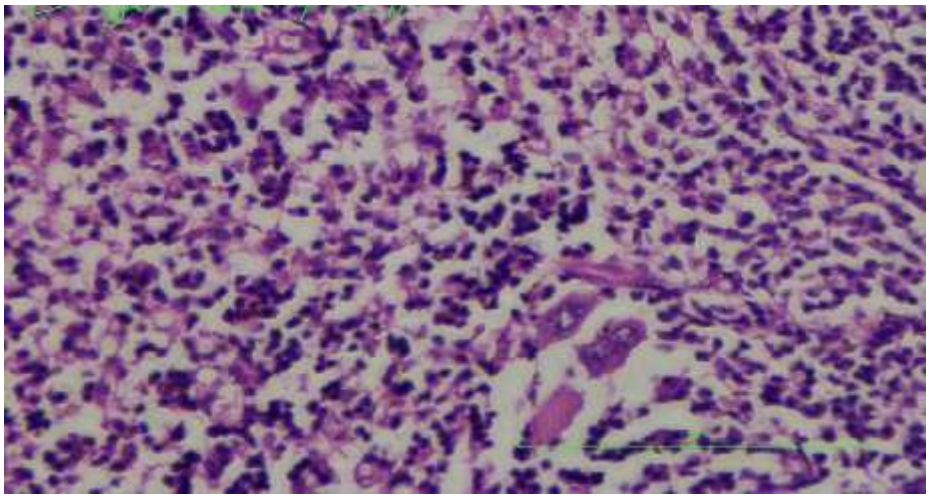

Figure 7 Histological analysis of spleen after administration of Positive control (Cyclosporine) 100«/kg showing decreased cellularity, trabecular and capsular changes observed at 400X.

DISCUSSION

Colchicine has been used for the treatment of biliary and liver cirrhosis (Sabouraud *et al*. 1992). Colchicine administration blocked CappD-crystal and urate crystal activated inflammation. Inflammation occurs due to prostaglandin E1 (PGE1).This shows that colchicine plays its role as anti-PG agent (Denko 1975).

In present study, the effect of colchicine was investigated after performing haemagglutination assay, mice lethality assay and Jerne plaque formation assay. Haemagglutination was performed to investigate its effect on antibody mediated immunity. B cells interact with the antigen and antibody secreting cells are formed. The antibodies formed from antibody secreting cells attach with antigen and counteract its effect (Ramanatha *et al*. 1995; Takuo *et al*. 1982). B cells are responsible for formation of antibodies and cytokines (Mauri and Bosma 2012). Humoral immunity can be investigated by using HA titer. B cells are involved in production of antibodies. (Gokhale*et al*. 2003).The present study has shown that gradual increase in dose of colchicine caused reduction in HA titer of antibodies present in serum of mice. Reduced HA titer of all doses of colchicine recommended decreased formation of IgM and IgG antibodies in serum of mice against SRBCs.

Mice lethality assay is used to investigate serological responses in mice prior immunization by vaccines and mortality ratio is determined (Ramanatha *et al*. 1995; Rishi *et al*. 2002). *Pasteurellamultocida* is disease causing agent and it spreads through airway. Mice were vaccinated with haemorrhagic septicemic vaccine prior to challenge with *Pasteurellamultocida* culture. Vaccine has ability to produce antibodies. Survival of mice was dependent on the capability of drug to form antibodies. If enough antibodies are produced to counteract antigen then mice can survive. If sufficient antibodies are not produced than mice could not survive (Ismail and Asad 2009). Immunization with vaccine is completed before injection of bacteria and mortality ratio is determined. Humoral immunity is produced with administration of vaccine (Finco *et al*. 2001). In this study, effect of colchicine was observed. There was death of all the mice in the non-vaccinated group within 24 hour period. There was no death of mice in the negative control. At 40µg/kg dose of colchicine, there was 50% mortality ratio. There was 75% mortality observed at 80µg/kg dose of colchicine. Maximum mortality was observed at the 160µg/kg colchicine dose i.e. 100%. The mortality observed in colchicine treated groups showed that with increasing dose mortality ratio was increased. With increasing dose of colchicine, antibody formation was reduced resulting in more mortality ratio at higher doses.

Spleen is lymphoid organ which has its role both in humoral and cellular responses. As it has effective potential in the humoral immune system, it can stimulate the antibody secreting cells to produce antibodies. These antibody secreting cells are present in lymphoid organ (Tiwari *et al.* 2004).In present study; the effect of colchicine was studied on plaque formation. Dose dependent decrease in antibody producing plasma cell was observed in colchicine administered groups as compared to negative control group. There was reduction in number of plaques in colchicine treated groups to negative control. Antibody formation was decreased with increasing the dose of colchicine. Hemolytic plaque was formed with antibody producing cell in center. It was believed that IgM secreting type antibodies were responsible for the formation of plaques. It was due to property of IgM that it can attach with high competence to complement (Ali *et al*. 2008).

In current study, colchicine showed significant reduction on body weight of mice with increasing dose of

colchicine. There was also no significant effect on weight of thymus but only there was significant reduction in difference of weight of spleen. There was reduction in spleen weight with increasing dose of colchicine at different groups from negative control. The present study proved that there were no significant changes in histopathology of thymus and spleen. At higher dose, increase in thickness of trabecular was seen. There was also presence of edema in the spleen at higher dose. Mild to moderate changes were observed in red pulp and white pulp ratio. Few histopathological changes of thymus were also observed mild atrophy and capsular changes were observed at higher dose. There were no significant histopathological changes at lower and medium doses in thymus and spleen of mice. Colchicine can be used safely at therapeutic doses because it has only side effects at high dose. It can be concluded from present study that there was dose dependent suppression of humoral immunity by administration of colchicine.

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