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Evaluation of Ketoprofen Effects on Humoral Immunity and Immune Organs in Mice

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Abstract.- The present project was designed to study the effects of ketoprofen on humoral immunity and to evaluate the toxic effects of ketoprofen on immune organs of mice. Effects of ketoprofen on humoral component of immune system were evaluated at two doses 1mg/kg and 5mg/kg by performing haemagglutination assay, and mice lethality test. Spleen and thymus were removed from the mice and histopathological examination was performed to observe the toxic effects of ketoprofen on these organs. Ketoprofen treatment exhibited significant ($p<0.001$) decrease in serum anti-SRBCs antibody titer as compared to the control. Mortality ratio was significantly ($p<0.001$) increased in ketoprofen treated mice. There was a significant ($p\leq0.01$) decrease in the weight of spleen and thymus in ketoprofen treated groups. Histopathological analysis of spleen of the mice treated with .ketoprofen presented slight increase in the thickness of the trabecular due to odema in spleen. Moreover, few medullary changes in the form of mild medullary atrophy occurred in the thymus of mice. These results provide the information that ketoprofen has immunosuppressive effects on humoral immunity therefore it can be used in patients with autoimmune diseases and to prevent allograft rejection.

Key words: Ketoprofen, immunomodulatory, humoral immunity, immune organs.

INTRODUCTION

Ketoprofen a NSAID was synthesized in France. It possess analgesic, antipyretic and anti-inflammatory properties (Celebi *et al*., 2009; Lu *et al*., 2004; Shinkai *et al*., 2008). Mostly it is used for joint pains, tissue injury and laminitis (Kawai *et al*., 2010). It is now used in 80 countries including USA for treatment of osteoarthritis and rheumatic diseases. It is therapeutically equal to indomethacin, aspirin and ibuprofen in the treatment of rheumatic diseases and is equivalent to aspirin for osteoarthritis treatment. Ketoprofen has a simple metabolism, short half-life and broad therapeutic window (Kantor, 1986).

Humoral immunity is antibody mediated immunity, which may be actively or passively acquired. It involves the participation of B-cells. The helper T-cells assist with B-cells and secrete B-cell growth factors, cytokines, and cause multiplication of B-cells. T-cells produce B-cell differentiation factors when B-cells count increases. B-cell differentiation factors transform B-cells into plasma cells which start producing anti-bodies (McCullough and Summerfield, 2005; Miriam and Kathryn, 2005). Different experimental models use haemagglutination assay to assess the effects on humoral immune system in the mice. A rise or decrease in the circulating titer demonstrate an increase or decrease in humoral immune response. Similarly, mice lethality test was used in different experiments to assess the effects on humoral immune response (Shendige *et al*., 2010).

In this study we investigated the effects of ketoprofen on humoral immune responses by performing haemagglutination assay and mice lethality test. Ketoprofen therapeutically effective analgesic and anti-inflammatory doses 1mg/kg/day and 5mg/kg/day were used for investigation. The effects of ketoprofen on immune organs such as spleen and thymus were also examined by performing histopathological analysis of these organs to analyze toxicity of ketoprofen.

MATERIALS AND METHODS

Animals

Five-to-seven week-old albino mice were purchased from Veterinary Research Institute (VRI),

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Lahore and kept in animal house of the University of Veterinary and Animal Sciences, Lahore, by taking into consideration all possible hygienic measures. The mice were housed in comfortable cages (5 to 7 mice per cage) and maintained on standard pellet diet and water *ad libitum* during the entire trial period. All experimental manipulations were undertaken in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals.

Doses and exposure schedules

After one week (for acclimatization) three groups of mice (five to seven mice per group) were treated with two doses of ketoprofen and PBS (control group). Ketoprofen was purchased from Sigma Aldrich (catalogue No. k1751-5G). Mice in the test group were administered ketoprofen intraperitoneally at dose rate of 1mg/kg and 5mg/kg. Antigens (SRBCs) and *Pasteurella multocida* for correspondent mice groups were injected 6 h after the daily injection of ketoprofen.

Haemagglutination assay (HA)

Mice were weighed and given ketoprofen 1mg/kg/day and 5mg/kg/day intraperitoneally for 28 days (5days a week). The control mice were given phosphate buffer saline only. Each mouse was immunized with 0.5×10^8 sheep red blood cells (SRBCs) intraperitoneally, including control mice. Blood samples were collected from each mouse at the end of the drug treatment and serum was separated. The antibody titers against the SRBCs were determined in the Ketoprofen treated and control mice by haemagglutination assay (Hassan *et al*., 2004).

Mice lethality test

Mice in ketoprofen treated groups were treated with 1mg/kg/day and 5mg/kg/day ketoprofen for 21 days. On the 7th and 17th day of the treatment, the animals were immunized with hemorrhagic septicemia vaccine (HS vaccine). On the 21st day, mice were challenged subcutaneously with 0.2 ml (25 lethal dose50) of the *Pasteurella multocida* culture containing 10^7 cells per ml. The animals were observed for a period of 72 hours to detect any mortality in the inoculated mice. The mortality ratio was obtained as described by (Bharani *et al*., 2010).

$$\text{Mortality ratio} = \frac{\text{No. of animal dead}}{\text{Total No. of animals}} \times 100$$

Effects on immune organ and histopathological examinations

Mice were weighed and given ketoprofen 1mg/kg/day and 5mg/kg/day intraperitoneally for 28 days. At the end of the 28th day, all mice were sacrificed by cervical dislocation. Spleen and thymus (TM) of each mouse were then collected and weighed. For histopathological examinations 10 % formaline was used to preserve the spleen and thymus. 5-µm sections of these tissues were stained with hematoxylin–eosin. Histopathological changes in spleen and thymus were analyzed and observed under light microscope and scored according to the degrees of changes (Hassan *et al*., 2004).

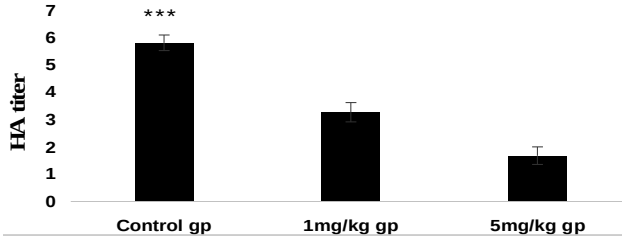

Fig. 1. Log 2 value of haemaglutination assay titer. ***p<0.001 compared to control.

RESULTS

Effect on humoral immune response

Ketoprofen treatment exhibited significant ($p<0.001$) decrease in serum anti-SRBCs antibody titer as compared to the control group. Ketoprofen at 5mg/kg showed the lowest HA titer with significant difference ($p<0.001$) from the control group. HA titer was comparatively more reduced in 5mg/kg ketoprofen treated group as compared to 1mg/kg ketoprofen treated group, but there was no statistical difference in HA titer between 1mg/kg and 5mg/kg ketoprofen treated groups (Fig. 1).

Mice lethality test was also performed to evaluate the effects of ketoprofen on the humoral

Table I.- Percentage mortality and organ weight of vaccinated control group and Ketoprofen treated groups.

	Control		Treated	
	Non-vaccinated (n=5)	Vaccinated (n=5)	1 mg/kg (n=5)	5 mg/kg (n=5)
Mortality after 24 h	5	0	2	5
Mortality after 48 h	0	1	1	0
Mortality after 72 h	0	0	0	0
Mortality (%)	100	20	60	100
Body weight before treatment (g)		26.98±2.69	27.42±3.91	27.9±2.44
Body weight after treatment (g)		27.94±2.52	27.64±3.94	27.58±4.86
Spleen weight (g)		0.18±0.07	0.16±0.02	0.13±0.05**
Spleen weight (% of BW)		0.64±0.22	0.56±0.13	0.44±0.19***
Thymus weight (g)		0.19±0.03	0.19±0.04	0.17±0.05
Thymus weight (% of BW)		0.69±0.15	0.67±0.20	0.64±0.15

Data are shown as mean ± SD (n=5) **p≤0.01 ***≤0.005

immunity. Administration of *pasteurella* culture produced 100 % mortality within 24 h in mice which were not vaccinated. The control group in which the mice were vaccinated with HS vaccine, only one mouse died with a mortality ratio of 20%. Highest mortality ratio was observed with 5mg/kg ketoprofen treated group where all mice died within 24 h after the administration of *pasteurella* culture. Mortality ratio was 60% in mice treated with ketoprofen 1mg/kg (Table I).

Effect on Immune organ weight

Individual mice body weight was measured before the start of experiment and after 28 days (5 days a week) treatment of ketoprofen. Body weight of the mice was slightly increased compared to the body weight of mice before treatment in control and 1mg/kg ketoprofen treated group but there was a little decrease in body weight of mice in 5mg/kg ketoprofen treated group compared to the start of the treatment. However, this difference was not statistically significant. There was a significant ($p \leq 0.01$) decrease in spleen weight of mice in 5mg/kg ketoprofen treated group compared to control and 1mg/kg ketoprofen treated group, similarly there was significant ($p \leq 0.005$) decrease in spleen weight % of BW. The weight of thymus was reduced in 5mg/kg ketoprofen treated group compared to 1mg/kg and control group however this difference was not statistically significant. Similarly thymus weight % of BW was reduced in 5mg/kg ketoprofen treated group compared to control and 1mg/kg ketoprofen treated group (Table I).

Histopathological assay

There were no changes observed in the spleen and thymus of mice in the control group. Histopathological analysis of spleen of the mice treated with 5mg/kg per day of ketoprofen showed a slight increase in the thickness of trabecular due to odema in the spleen. Odematous fluid was seen in cortex and medulla but it was more prominent near the trabecular sheet. Mild to readily detectable lymphocytic (white pulp) atrophy was observed but there was no significant difference seen in red and white cell ratio (Fig. 2).

Histopathological analysis of the thymus of mice treated with 5mg/kg of ketoprofen presented a few medullary changes in the form of mild medullary atrophy in the thymus of mice. No other histological changes were observed in the thymus of mice treated with 5mg/kg of ketoprofen (Fig. 3).

No significant changes were observed in the histopathological analysis of spleen and thymus in mice treated with 1mg/kg per day of Ketoprofen. However, some odematous fluid causing the odema in spleen was observed in histopathological observation. Moreover, only a few changes were seen in the trabecular of spleen (Table II).

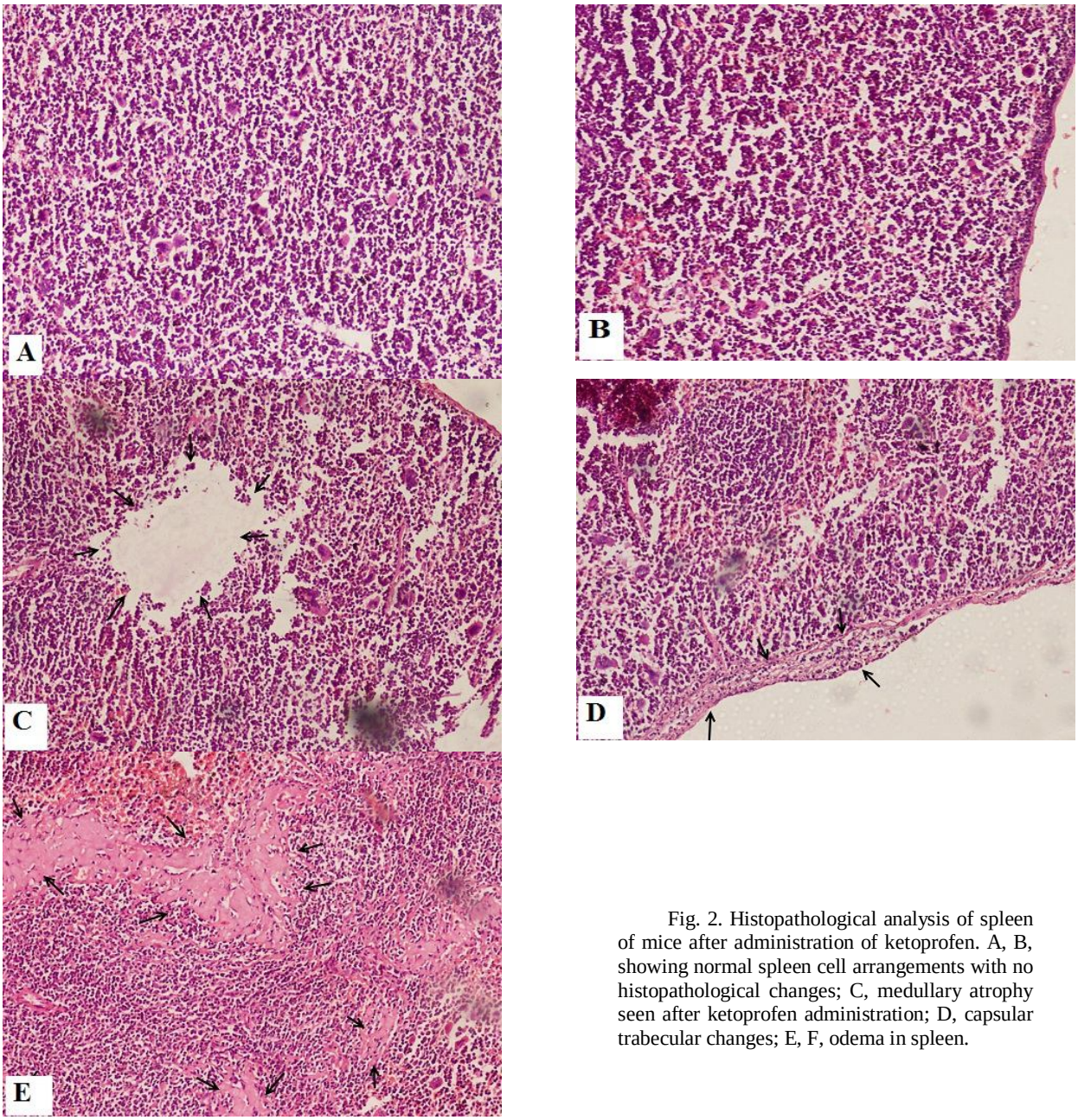

Fig. 2. Histopathological analysis of spleen of mice after administration of ketoprofen. A, B, showing normal spleen cell arrangements with no histopathological changes; C, medullary atrophy seen after ketoprofen administration; D, capsular trabecular changes; E, F, odema in spleen.

DISCUSSION

In the present study the effects of ketoprofen on humoral component of immune system were evaluated at therapeutically analgesic and anti-inflammatory doses range in mice by performing heamagglutination assay and mice lethality test. Similarly effects on immune organ of mice were analyzed.

Humoral immunity is regulated by the production of antibodies against specific antigens (Marasco, 1997). HA titre is the direct measure to determine the level of humoral immunity. Serum immunoglobulin refers to a group of serum molecules produced by B-lymphocytes, they are soluble and are secreted from B-cell receptors and

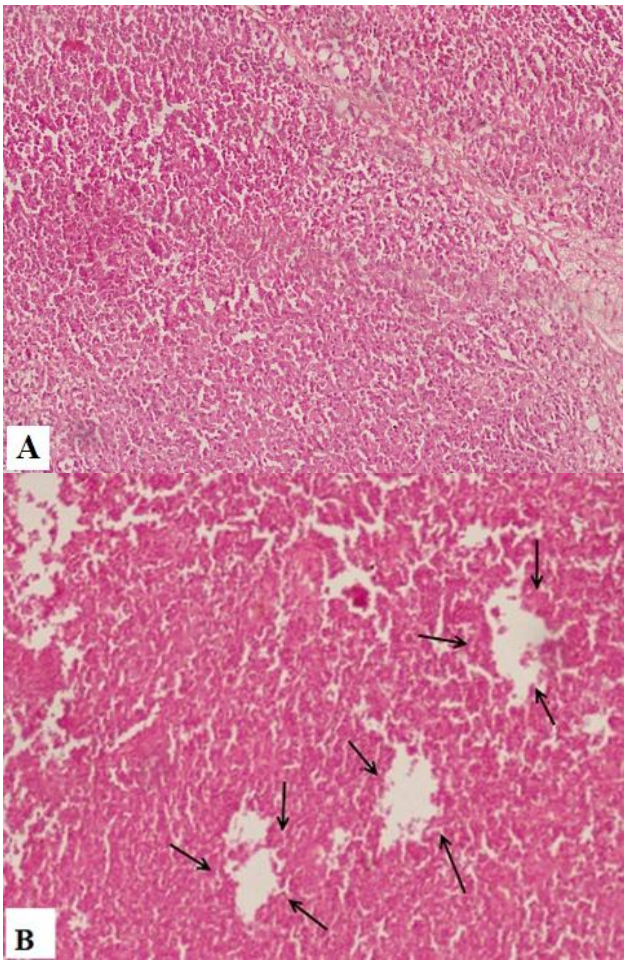

Fig.3. Histological analysis of thymus of mice after ketoprofen administration. A, normal thymus cell with no changes seen in control group; B shows medullary atrophy after 5mg/kg administration of ketoprofen.

are produced to a maximum level to counter the invasion by an antigen, and hence they are also called antibodies (Ismail and Asad 2009). In HA titer antibodies bind with antigen to neutralize them (Takuo and Keith, 1982). The results of our study exhibited that ketoprofen at a high dose has significantly decreased HA titer. Decreased HA titer by the highest dose of ketoprofen suggested the decreased production of IgG and IgM antibodies in the serum of mice against sheep's red blood cells. No other significant data is available regarding ketoprofen effects on HA titer.

Mice lethality test determines the strength of mice to survive against the antigen. It involves the immunization of mice prior to the administration of an antigen (Rishi *et al*., 2002). In the present study ketoprofen showed 100% mortality ratio at dose 5mg/kg, 60% at dose 1mg/kg and 20% mortality ratio was observed in the control group. Results of the mice lethality test performed in the present study indicated the decreased production of antibodies against pasteurella multocida by B-lymphocytes of mice immunized with hemorrhagic septicemia vaccine at the highest dose of ketoprofen administered (5mg/kg). Results of the present study suggest that ketoprofen has significantly suppressive effects on humoral immunity.

Table II.- Histopathological changes in the spleen and thymus of mice treated with different doses of ketoprofen.

	Treated	
	1 mg/kg	5 mg/kg
Control	-	-
White pulp atrophy	-	-
Capsular-trabecular changes	-/+	+
Odema in spleen	+	+++
Red pulp/white pulp ratio changes	-	-
Medullary atrophy in thymus	-	+
Starry sky appearances in thymus	-	-
Capsular and trabecular change in thymus	-	-

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

Our study showed no significant effect on mice weight after administration of ketoprofen, however spleen weight was reduced after 5mg/kg ketoprofen administration. There was no effect on weight of thymus after ketoprofen administration. No other data is available regarding the ketoprofen effects on mice body weight and immune organs.

Correspondingly results of our study presented that ketoprofen did not show significant changes in histopathological analysis of immune system at 1mg/kg treated group but at 5mg/kg ketoprofen treated group odema in spleen was observed. No major histopathological or functional changes in the immune system of mice were observed with ketoprofen administration. Ketoprofen can be safely used as it does not cause toxicity to the immune organs (Forrest *et al*., 2002).

Some other studies have shown that ketoprofen ameliorate lymphatic vascular insufficiency and odema can be observed in the histopathological examinations (Nakamura *et al*., 2009).

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Conflict of interest

The authors declare that there is no conflict of interest.

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