IV. RESULTS

The pharmacological studies and safety evaluation of HF cross bred cow urine and routine comparative study of urine from different breeds of cattle were carried out in the present investigation.

4.1 Pharmacological studies

4.1.1 Analgesic activity

4.1.2 Male rats

Reaction time in seconds in treatment group II, III, IV and V were almost similar at all intervals when compared to control group I. But when compared to standard control i.e., group VI ibuprofen administered rats, the reaction times were showing significant difference at (P<0.01, P<0.001). In group VI, at 1h the reaction time 3.54±0.40, at 2 h, the reaction time was 3.49±0.33, at 3h the reaction time was 3.08±0.19 and at 4 h the reaction time was 3.15±0.48 seconds. There was significant increase in reaction time in standard control group (P<0.001). There was no significant difference between control and cow urine treated groups (Table 1 and Fig. 1).

4.1.3 Female rats

Reaction times in treatment group VII, VIII, XI, X and XI group were almost lesser when compared to reference drug Ibuprofen i.e., standard control group XII. In standard control XII (Ibuprofen administered group at 1h reaction time was 4.53±1.77 at 2 h the reaction time was 3.27±0.26 at 3h the reaction time was 3.02±0.19 and at 4 h the reaction time was 3.61±0.32. There was significant increase in reaction time in standard control group (P<0.001) (Table 2 and Fig. 2).
4.2 Antipyretic activity

4.2.1 Male rats

Body temperature in male rats in group I, II, III, IV, V and VI at -18 hours were 36.65±0.13, 36.43±0.14, 36.43 ±0.26, 36.50 ±0.23, 36.44±0.25 and 36.23 ±0.19 °C respectively and just before the drug administration i.e., 0 h in group I, II, III, IV, V and VI the temperature was 38.12 ±0.18, 38.17 ±0.10, 38.08 ±0.17, 38.30 ±0.23, 38.42 ±0.16 and 38.34 ±0.14 °C respectively.

In group II in paracetamol treated standard control groups, the temperature at 1 h, 3 h, 5 h, 18 h, and 24 h were 37.41 ±0.10, 36.48 ±0.19, 36.38 ±0.14, 36.50 ±0.20 °C and 36.25 ±0.14 °C, respectively (Table 3 and Fig. 3).

There was significant decrease in body temperature in standard control group (P<0.001) IV at 3 hrs.

4.2.2 Female rats

Body temperature in female rats in group VII, VIII, XI, X, XI and XII at -18 hours were 36.79±0.03, 36.80±0.06, 36.50±0.13, 36.53±0.13, 36.60±0.15 °C and 36.67±0.12 °C, respectively and just before the drug administration that is, 0 h in group VII, VIII, XI, X, XI and XII the temperature were 38.29±0.11, 38.34±0.16, 38.28±0.17, 37.75 ±0.12, 38.11 ±0.13 °C and 37.81 ±0.14 °C, respectively.

In group VIII paracetamol treated standard control groups the temperature at 1 h, 3h, 5h and 18h were 37.08±0.17, 36.55±0.02, 37.20±0.11 and 36.52±0.06 °C, respectively (Table 4 and Fig. 4).

There was significant decrease in body temperature in standard control group (P<0.001).
There was no significant decrease in body temperature in treatment groups (P<0.05) in both sexes.

4.3 Anti-inflammatory activity

4.3.1 Acute anti-inflammatory activity of cow urine in male rats

The percentage thickness in the right paw in the control group was 180.75±0.75 whereas in treatment groups II, III, IV and V were 134.88±10.44, 176.27±6.5, 165.95±7.6 and 167.42±11.63, respectively. In group VI i.e., standard control diclofenac administered group, the percentage thickness was 177.01±10.07. (Table 5 and Fig. 5). There was no significant (P<0.05) decrease in the inflammation in urine treated groups.

4.3.2 Chronic anti-inflammatory activity in male rats

The percentage thickness in the right paw, six days after drug administration in the groups I, II, III, IV and V were 152.80±4.19 148.70±10.51, 153.92±7.11, 149.03±10.47 and 147.77±8.15, respectively. In standard control group i.e., diclofenac administered group VI it was 112.52±2.85.

There was significance decrease in paw thickness in standard control group at P<0.01 (Table 6 and Fig.7).

4.3.3 Acute anti-inflammatory activity in female rats

The percentage thickness in the right paw in the control groups was 172.96±3.84. In group VIII, IX, X and XI was, 189.37±9.39, 179.93±7.50, 167.95±8.56 and 170.90±4.73, respectively. In group XII i.e., standard control diclofenac administered group was 155.03±9.14 (Table 7 and Fig.6). There was no significant (P<0.05) decrease in the inflammation in treatment groups.
4.3.4 Chronic anti inflammatory activity in female rats

The percentage thickness in the rat paw six days after drug administration in the groups VII, VIII, IX, X and XI were 149.69±7.01, 157.02±6.67, 147.68±11.42, 137.71±11.47 and 141.54±8.01, respectively. In standard control group XII it was 105.73±0.89.

There was significance difference decrease in paw thickness in diclofenac treated group at P<0.01 (Table 8 and Fig.8).

4.4 Hepatoprotective activity

4.4.1 Male rats

4.4.1.1 Biochemical parameters

4.4.1.1.1 Bilirubin

In groups I, II, III, IV, V VI and VII the concentration of bilirubin were 0.51±0.02 mg/dl, 2.58±0.17 mg/dl, 0.06±0.03 mg/dl, 0.99±0.19 mg/dl, 1.11±0.18 mg/dl, 1.22±0.32 mg/dl and 1.05±0.09 mg/dl, respectively. (Table 9 and Fig. 9).

4.4.1.1.2 AST (IU/l)

In groups I, II, III, IV, V, VI and VII the AST concentration were 69.67±1.63 IU/l, 189.33±1.94 IU/l, 96.00±1.53 IU/l, 109.83±5.23 IU/l, 108.00±3.51 IU/l, 110.33±3.28 IU/l and 118.16±4.22 IU/l, respectively. There was significant decrease in reference drug treated group and urine treated groups at P<0.01 (Table 9 and Fig. 11).

4.4.1.1.3 ALT (IU/l)

In groups I, II, III, IV, V VI and VII the ALT concentration were 49.17±1.80 IU/l, 106.50±2.89 IU/l, 62.50±2.99 IU/l, 72.66±1.89 IU/l, 74.83±2.90 IU/l,
79.00±1.69 IU/l and 75.67±2.62 IU/l, respectively. There was significant difference between all the group There was significant decrease in reference drug treated group and urine treated groups at P<0.01 (Table 9 and Fig. 13).

4.4.1.1.4 Total protein (g/dl)

In groups I, II, III, IV, V, VI and VII, the total protein concentration were 7.61±0.24 g/dl, 4.28±0.29 g/dl, 8.23±0.25 g/dl, 6.77±0.33 g/dl, 5.95±0.23 g/dl, 5.79±0.15 g/dl and 6.19±0.31 g/dl respectively (Table 9 and Fig.15).

4.4.1.1.5 ALP (IU/l)

In groups I, II, III, IV, V and VI the total alkaline phosphatase concentration were 129.67±1.50 IU/l, 309.17±2.39 IU/l, 146.33±2.03 IU/l, 170.33±6.40 IU/l, 183.00±6.87 IU/l, 185.16±6.76 IU/l and 190.33±6.33 IU/l, respectively. There was significant decrease in reference drug treated group and urine treated groups at P<0.05, P<0.01 (Table 9 and Fig. 17).

4.4.2 Female rats

4.4.2.1 Biochemical parameters

4.4.2.1.1 Bilirubin (mg/dl)

In groups VIII, IX, X, XI, XII, XIII and XIV the concentration of bilirubin were 0.76±0.148 mg/dl, 1.02±0.180 mg/dl, 1.43±0.130 mg/dl, 1.46±0.312 g/dl and 1.52±0.007 g/dl, respectively. There was significant decrease in reference drug treated group and urine treated groups (P<0.05, P<0.01) when compared to CCl₄ treated groups (Table 10 and Fig. 10).
4.4.2.1.2 AST (IU/l)

In groups VIII, IX, X, XI, XII, XIII and XIV AST concentration were 189.33±1.94 IU/l, 96.00±1.52 IU/l, 124.50±7.53 IU/l, 117.00±9.00 IU/l, 112.16±14.82 IU/l and 113.00±10.77 IU/l, respectively. There was significant decrease in reference drug treated group and urine treated groups at P<0.05, P<0.01 (Table 10 and Fig. 12).

4.4.2.1.3 ALT (IU/l)

In groups VIII, IX, X, XI, XII, XIII and XIV, ALT concentration were 49.16±1.79 IU/l, 108.66±2.51 IU/l, 62.50±2.98 IU/l, 64.33±2.81 IU/l, 69.33±1.56 IU/l, 67.00±3.37 IU/l and 64.00±3.75 IU/l, respectively. There was significant decrease in reference drug treated group and treatment groups at P<0.01 (Table 10 and Fig. 14).

4.4.2.1.4 Total protein (g/dl)

In groups VIII, IX, X, XI, XII, XIII and XIV the total protein concentration were 7.61±0.24 g/dl, 4.28±0.29 g/dl, 8.23±0.25 g/dl, 6.35±0.25 g/dl, 6.01±0.28 g/dl, 5.96±0.26 g/dl and 5.83±0.2 g/dl, respectively. There was significant increase in reference drug treated group and urine treated groups when compared to CCl₄ treated group (P<0.05, P<0.01) (Table 10 and Fig. 16).

4.4.2.1.5 Alkaline phosphatase

In groups VIII, IX, X, XI, XII, XIII and XIV the total alkaline phosphatase concentration was 129.66±1.49, 309.16±2.38 IU/l, 146.33±2.02 IU/l, 168.66±4.91 IU/l, 185.16±12.87 IU/l, 176.16±8.56 IU/l and 175.33±7.61 IU/l, respectively. There was significant decrease in reference drug treated group and urine treated groups at P<0.05 and P<0.01 (Table 10 and Fig. 18).
4.4.3 Pathology

4.4.3.1 Gross pathology: Liver – hemorrhage and congestion, oedema in CCl₄ group.

4.4.4 Histopathology of liver in male rats

In control animals section of liver was showing normal architecture. In CCl₄ treated male rats showing severe fatty changes of liver with fatty vacuoles, fatty cysts degeneration, necrosis and also hepatocytes with infiltration of inflammatory cells. In CCl₄ with silymarin treated male rats liver was showing mild hepatocytes degeneration and mild degree of necrosis. In CCl₄ with different doses of urine in male rats showing normal arrangements of hepatocytes around the central vein and mild fatty change with infiltration of hepatocytes.

4.4.5 Histopathology of liver in female rats

In control animals section of liver was showing normal architecture. In CCl₄ treated male rats showing severe degeneration and necrosis of hepatocytes. In CCl₄ with silymarin treated female rats liver was showing mild degree of fatty degeneration and hepatocytes around the central vein. In CCl₄ with different doses of urine in female rats liver showing mild degree of fatty degeneration bile duct epithelial hyperplasia and congestion of vessels.

4.5 Wound healing property of cow urine

4.5.1 Male rats

The percentage of wound in group I, II, III, IV, V, VI and VII on 2nd day was 80.9±2.4, 87.8±1.2, 78.7±2.0, 75.0±1.2, 75.9±1.9, 80.2±1.7 and 80.0±2.8, respectively.
The percentage of wound in group I, II, III, IV, V, VI and VII on 10th day was 6.5±0.9, 4.1±0.8, 5.4±0.6, 5.4±0.8, 5.4±0.8, 7.9±0.8 and 5.2±1.3, respectively.

The percentage of wound in group I, II, III, IV, V, VI and VII on 12th day was 1.4±0.3, 0.4±0.1, 2.0±0.1, 1.5±0.1, 1.2±0.2, 1.8±0.2, and 1.6±0.2, respectively.

The percentage of wound in group I, II, III, IV, V, VI and VII on 14th day was 0.0±0.0, 0.0±0.0, 0.3±0.2, 0.1±0.1, 0.1±0.1, 0.1±0.1, and 0.4±0.2, respectively (Table. 11 and Fig. 19).

4.5.2 Female rats

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 2nd day was 88.5±2.2, 83.9±3.9, 88.4±3.1, 84.4±3.9, 84.5±4.0, 84.3±3.8 and 83.6±4.4 in female rats.

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 4th day was 72.1±1.7, 65.6±3.2, 68.7±3.2, 64.2±3.1, 65.1±3.6, 63.1±2.9 and 60.7±2.7 in female rats. There was significant increase in the percentage of wound healing in external applied urine treated group (P<0.05).

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 12th day was 2.6±0.8, 2.9±0.6, 3.8±0.6, 3.3±0.7, 3.8±1.0, 2.3±0.8 and 0.0±0.0 in female rats. There was significant increase in percentage of wound healing in externally applied urine treated group at P<0.01.

The percentage of wound area in group VIII, IX, X, XI, XII, XIII and XIV on 14th day was 0.4±0.4, 0.5±0.3, 0.6±0.2, 0.5±0.3, 0.5±0.3, 0.0±0.0 and 0.0±0.0 in female rats. (Table 12 and Fig. 20).
4.6 Immunotoxicity study

4.6.1 Parameters of humoral immune response

4.6.1.1 Haemagglutination test

The antibody titers of treated and control groups of male and female rats are given in Table 13 and Fig. 21 and 22. There was no significant difference found between control and treated groups at P>0.05.

4.6.1.2 Total serum immunoglobulin concentration

The total serum immunoglobulin concentrations of treated and control groups of male and female rats are given in Table 14 and Fig. 23 and 24.

The TIG concentration of treated group of both male and female rats did not differ significantly (P>0.05) from the respective control group TIG concentration.

4.6.1.3 Total serum protein concentration

The total serum protein concentrations of treated and control groups of male and female rats are given in Table 16. The TSP concentration of treated group of both male and female rats did not differ significantly (P>0.05) from the respective control group TSP concentration.

4.6.2 Parameters of cell mediated immune response

4.6.2.1 Total leukocytes count

The total leukocyte count of male and female treated and control group rats is given in Table 18. The total leukocyte count of treated groups did not differ significantly (P>0.05) from the respective control group.
4.6.2.2 Absolute lymphocyte count

The ALC of male and female treated and a control group rat was given in Table 16. The absolute lymphocyte count of treated groups did not differ significantly (P>0.05).

4.6.2.3 Dinitro chlorobenzene (2, 4, -DNCB) skin sensitivity test

The skin thickness in treated and control group is given in Table 17 and Fig. 25 and 26).

4.7 Acute toxicity study

4.7.1 Observation of clinical signs

There were no observable clinical signs. Cow urine did not result in mortality in either male or female rats even at the highest dose of 5 ml/kg body weight tested. Further LD\(_{50}\) value was also not determined.

4.8 Sub-acute toxicity study (Repeated dose 28 day oral toxicity study)

4.8.1 Observation of clinical signs

There were no observable clinical signs found.

4.8.1.1 Body weight

The rats were weighed individually at the beginning of the study and at weekly intervals till the end of the study. The body weights of male and female rats are given in Table 19 and 20, Fig. 27 and 28).

In group II, group III, group IV and group V in male on day 7, the body weight were 165.33±3.93, 166.50±2.86, and 168.66±2.38 and 169.33±2.96g, respectively. Group V value was 183.83±4.81 g.
In group II, group III, group IV and group V in male on day 14, the body weight were 177.17±4.49, 177.83±2.24, and 180.33±3.20 and 180.50±1.72g, respectively. The group V value was 194.17± 4.63 g.

In group II, group III, group IV and group V in male on day 21, the body weight were 189.50±4.12, 188.33±1.61, 189.83±2.88 and 188.00±2.73g, respectively. Group V value was 207.00 ± 5.01 g.

In group II, group III, group IV and group V in male on day 28, the body weight were 197.66±3.65, 197.33±1.60 and 199.16±2.30 and 199.00±2.60g, respectively. Control group value was 215.83 ± 4.54 g.

In group VII, group VIII, group IX and group X in female on day 7, the body weight were 168.50±4.34, 167.33±4.69, 167.66±2.84 and 163.33±3.43g, respectively. Group X value was 181.83± 3.57 g.

In group VII, group VIII, group IX and group X in female on day 14, the body weight were 181.33±4.03, 176.67±5.89, 178.16±2.90 and 175.16±2.46g, respectively. Group X value was 197.50± 3.91 g.

In group VII, group VIII, group IX and group X in female on day 21, the body weight were 192.50±4.59, 189.66±4.60, 190.33±2.29 and 186.50±1.60g, respectively. Group X value was 211.17± 3.40 g.

In group VII, group VIII, group IX and group X in female on day 28, the body weight were 208.00±5.17, 205.33±5.05, 204.33±4.04 and 197.00±2.32g, respectively. Control group value was 223.83± 3.23 g. There was no significant difference between control and treatment groups.
4.8.2 Hematology

The hematological values like TEC, Hct, Hb, MCV, MCH, MCHC, Neutrophils, TLC, Lymphocyte, Monocyte and Eosinophils were estimated here.

4.8.2.1 Male and female rats

4.8.2.1.1 TEC

In group II, group III, group IV and group V in male on day 14, the TEC was 7.92±0.30, 7.22±0.34, 6.30±0.19 and 6.54±0.14 x 10^6/mm^3, respectively. This was no significant decrease when compared to control group 7.34±0.23 x 10^6/mm^3.

In group II, group III, group IV and group V in male on day 28, the TEC was 7.67±0.27, 6.04±0.24, 5.86±0.23 and 5.73±0.22 x 10^6/mm^3, respectively. Control group value was 7.51±0.29 x 10^6/mm^3.

In group VII, group VIII, group IX and group X in female on day 14, the TEC was 7.82±0.24, 7.72±0.22, 7.63±0.15 and 7.15±0.31 x 10^6/mm^3, respectively. Control group value was 7.74±0.24 x 10^6/mm^3.

In group VII, group VIII, group IX and group X in female on day 28, the TEC was 7.79±0.16, 7.20±0.16, 6.61±0.21 and 6.58±0.30 x 10^6/mm^3. Control group value was 7.74±0.25 x 10^6/mm^3, respectively (Table 21 and Fig. 29 and 30). There was significant difference between treated and control group.

4.8.2.1.2 Haematocrit

In group II, group III, group IV and group V in male on day 14, the Hct were 39.94±0.13, 38.16±1.63, 36.44±1.09 and 35.84±0.32 %, respectively. Control group value was 40.20±0.59%.
In group II, group III, group IV and group V in male on day 28, the Hct were 40.44±0.25, 35.62±0.95, 34.24±0.59 and 30.58±0.72, respectively. Control group value was 37.93±1.22 %.

In group VII, group VIII, group IX and group X in female on day 14, the Hct were 39.61±0.38, 38.98±1.18, 35.11±0.81 and 36.96±1.06 %, respectively. Control group value was 39.52±0.29 %.

In group VII, group VIII, group IX and group X in male on day 28, the Hct were 38.33±0.77, 34.69±0.82, 34.23±0.59 and 32.67±1.25 %, respectively. Control group value was 39.27±0.34 % (Table 22 and Fig. 51 and 52). This was significant difference between treated and control group.

4.8.2.1.3 Hemoglobin

In group II, group III, group IV and group V in male on day 14, the Hb were 14.56±0.19, 13.81±0.25, 13.94±0.33 and 13.79±0.19 g%. Control group value was 16.37±0.57 g%.

In group II, group III, group IV and group V in male on day 28, the Hb were 14.68±0.21, 13.55±0.21, 13.29±0.16 and 13.26±0.18 g%. Control group value was 14.94±0.42 g%.

In group VII, group VIII, group IX and group X in female on day 14, the Hb were 14.48±0.23, 14.28±0.23, 13.92±0.19 and 13.78±0.30 g%. Control group value was 15.48±0.29 g%.

In group VII, group VIII, group IX and group X in female on day 28, the Hb were 14.23±0.29, 13.71±0.27, and 13.53±0.13 and 13.28±0.27 g%. Control group value was 15.10±0.38 g% (Table 23 and Fig. 33 and 34). This was significant difference between treated and control group.
4.8.2.1.4 MCV

In group II, group III, group IV and group V in male on day 14, the MCV were 53.47±0.34, 46.75±1.78, 48.73±0.19 and 46.09±1.69 fl. Control group value was 55.24±1.04 fl.

In group II, group III, group IV and group V in male on day 28, the MCV were 52.29±0.88, 48.52±1.29, 47.96±0.44 and 48.93±0.45 fl. Control group value was 54.04±0.31 fl.

In group VII, group VIII, group IX and group X in female on day 14, the MCV were 51.12±1.88, 52.51±1.77, 54.06±2.13 and 49.80±0.35 fl. Control group value was 54.33±0.37 fl.

In group VII, group VIII, group IX and group X in female on day 28, the MCV were 46.28±0.93, 49.02±1.62, 54.63±1.85 and 46.76±1.61 fl respectively. Control group value was 54.33±0.50 fl (Table 24 and Fig. 35 and 36). There was significant difference between treated and control group.

4.8.2.1.5 MCH

In group II, group III, group IV and group V in male on day 14, the MCH were 18.67±0.30 pg, 19.32±0.51 pg, 19.16±0.35 pg and 18.67±0.31 pg, respectively. Control group value was 20.69±0.47, respectively.

In group II, group III, group IV and group V in male on day 28, the MCH were 19.20±0.46 pg, 19.57±0.45 pg, 18.81±0.27 pg and 18.44±0.18 pg, respectively. Control group value was 20.96±0.43 pg.

In group VII, group VIII, group IX and group X in female on day 14, the MCH were 19.25±0.42, 19.25±0.56, 19.49±0.26 and 19.52±0.38, respectively. Control group value was 20.54±0.45.
In group VII, group VIII, group IX and group X in female on day 28, the MCH were 19.20±0.57, 18.76±0.46, 18.36±0.20 and 17.82±0.48, respectively. Control group value was 20.62±0.38 (Table 25 and Fig. 37 and 38). There was significant difference between treated and control group.

### 4.8.2.1.6 MCHC

In group II, group III, group IV and group V in male on day 14, the MCHC were 39.35±0.99, 36.67±1.39, 36.80±0.79 and 35.64±0.45 g/dl respectively. Control group value was 41.64±0.48 g/dl.

In group II, group III, group IV and group V in male on day 28, the MCHC were 37.60±0.83, 38.61±1.83, 36.53±0.41 and 32.86±0.96 g/dl respectively. Control group value was 39.06±0.33 g/dl.

In group VII, group VIII, group IX and group X in female on day 14, the MCHC were 39.28±0.85, 37.21±1.52, 35.93±0.87 and 36.81±1.36 g/dl respectively. Control group value was 41.41±0.34 g/dl.

In group VII, group VIII, group IX and group X in female on day 28, the MCHC were 38.19±0.75, 35.85±1.35, 34.70±0.66 and 32.39±0.91 g/dl respectively. Control group value was 39.69±0.47 g/dl (Table 26 and Fig. 39 and 40). There was significant difference between treated and control group.

### 4.8.2.1.7 Neutrophils

In group II, group III, group IV and group V in male on day 14, the neutrophil count were 22.87±0.76, 17.01±1.73, 17.61±0.29 and 18.24±1.83 % respectively. Control group value was 17.42±0.31 %.
In group II, group III, group IV and group V in male on day 28, the neutrophil count were 23.05±1.22, 17.40±0.69, 26.80±1.53 and 25.41±2.33 % respectively. Control group value was 17.30±0.24%.

In group VII, group VIII, group IX and group X in female on day 14, the neutrophil count were 20.34±0.48, 16.96±1.73, 17.13±1.74, and 16.63±1.34 % respectively. Control group value was 17.65±0.28 %.

In group VII, group VIII, group IX and group X in female on day 28, the neutrophil count were 20.03±0.62, 18.31±1.00, 17.19±0.77, and 18.04±0.89 % respectively. Control group value was 17.19±0.31 % (Table. 27 and Fig. 41 and 42). There was significant difference between treated and control group.

4.8.2.1.8 TLC

In group II, group III, group IV and group V in male on day 14, the TLC were 9.74±0.45, 8.72±0.55, 8.25±0.28 and 8.15±0.25 x 10³/mm³, respectively. Control group value was 8.46±0.23 x 10³/mm³.

In group II, group III, group IV and group V in male on day 28, the TLC were 8.38±0.38, 8.48±0.27, 7.85±0.13 and 6.78±0.14 x 10³/mm³, respectively. Control group value was 8.67±0.24 x 10³/mm³.

In group VII, group VIII, group IX and group X in female on day 14, the TLC were 8.47±0.36, 8.74±0.25, 8.57±0.31 and 7.73±0.13 x 10³/mm³, respectively. Control group value was 8.79±0.35 x 10³/mm³.

In group VII, group VIII, group IX and group X in female on day 28, the TLC as 8.09±0.33, 7.63±0.27, 7.90±0.17 and 7.28±0.25 x 10³/mm³ respectively. Control group value was 9.70±0.21 x 10³/mm³ (Table 28 and Fig. 43 and 44). There was significant difference between treated and control group.
4.8.2.1.9 Lymphocyte

In group II, group III, group IV and group V in male on day 14, the lymphocyte count were 74.08±1.81, 75.31±1.61, 75.92±1.09 and 74.60±1.23 %, respectively. Control group value was 78.58±0.29 %.

In group II, group III, group IV and group V in male on day 28, the lymphocyte count were 71.50±2.34, 72.91±1.17, 73.67±1.19 and 73.12±1.29 % respectively. Control group value was 79.27±0.46 %.

In group VII, group VIII, group IX and group X in female on day 14, the lymphocyte count were 72.38±1.13, 72.67±0.87, 71.67±1.69 and 75.60±1.91 % respectively. Control group value was 78.68±0.23 %.

In group VII, group VIII, group IX and group X in female on day 28, the lymphocyte count were 71.49±1.09, 70.64±1.42, 69.13±1.23 and 67.62±2.66 % respectively. Control group value was 79.27±0.46 % (Table 29 and Fig. 45 and 46). There was significant difference between treated and control group.

4.8.3 Monocyte

In group II, group III, group IV and group V in male on day 14 the corresponding monocyte were 1.06±0.10 %, 0.87±0.04 %, 1.52±0.13% and 1.44±0.11 respectively. A control group value was 1.29±0.10 %.

In group II, group III, group IV and group V in male on day 28, the monocyte were 0.78±0.14, 0.85±0.04, 1.59±0.03 and 1.47±0.06 %. Control value was 1.21±0.06 %.

In group VII, group VIII, group IX and group X in female on day 14 the monocyte were 1.03±0.08, 0.81±0.05, 1.11±0.04 and 1.14±0.05 % respectively. Control group value was 1.17±0.12 %.
In group VII, group VIII, group IX and group X in female on day 28, the monocyte were 0.91±0.05, 0.86±0.05, 0.90±0.07 and 0.76±0.12 % respectively. Control value was 1.22±0.06 % (Table 30 and Fig. 47 and 48). There was significant difference between treated and control group.

**4.8.3.1 Eosinophils**

In group II, group III, group IV and group V in male on day 14 the corresponding eosinophils were 0.99±0.13, 1.52±0.11, 1.18±0.01 and 1.20±0.01 %, respectively. Control group value was 1.02±0.10 %.

In group II, group III, group IV and group V in male on day 28, eosinophils were 1.06±0.14, 1.27±0.16, 1.16±0.01 and 1.16±0.01 %, respectively. Control value was 0.85±0.04 %.

In group VII, group VIII, group IX and group X in female on day 14 the eosinophils were 1.16±0.14, 1.34±0.15, 1.21±0.01 and 1.25±0.02 %, respectively. Control group value was 1.17±0.13 %.

In group VII, group VIII, group IX and group X in female on day 28, the eosinophils were 1.06±0.14, 1.30±0.16, 1.22±0.59 and 1.19±0.02 %, respectively. Control value was 1.18±0.17 % (Table 31 and Fig. 49 and 50). There was significant difference between treated and control group.
4.8.4 Biochemical parameters

4.8.4.1 Male and female rats

4.8.4.1.1 BUN

In group II, group III, group IV and group V in male on day 14, the BUN concentration were 22.06±0.52, 22.13±0.43, 20.72±0.16 and 22.36±0.31 mg/dl, respectively. Control group value was 21.89±1.10 mg/dl.

In group II, group III, group IV and group V in male on day 28, the BUN concentration were 23.10±0.91, 21.94±0.41, 21.30±0.23 and 23.21±0.55 mg/dl, respectively. Control group value was 23.73±0.92 mg/dl.

In group VII, group VIII, group IX and group X in female on day 14, the BUN 24.90±0.77, 26.36±0.00, 23.28±0.86 and 23.06±0.89 concentration was mg/dl respectively. Control group value was 20.96±0.84 mg/dl.

In group VII, group VIII, group IX and group X on day 28, the BUN concentration were 24.43±0.93, 25.16±0.78, 23.13±0.80 and 20.21±0.05 mg/dl, respectively. Control group value was 22.73±0.79 mg/dl (Table 32 and Fig. 51 and 52). There was significant difference between treated and control group.

4.8.4.1.2 Bilirubin

In group II, group III, group IV and group V in male on day 14, the Bilirubin concentration were 0.32±0.02, 0.19±0.03, 0.27±0.03 and 0.21±0.05 mg/dl respectively. Control group value was 0.34±0.02 mg/dl.

In group II, group III, group IV and group V in male on day 28, the Bilirubin concentration were 0.20±0.03, 0.20±0.03, 0.21±0.02 and 0.24±0.03 mg/dl, respectively. Control group value was 0.31±0.05 mg/dl.
In group VII, group VIII, group IX and group X in female on day 14, the Bilirubin concentration were 0.31±0.05, 0.34±0.08, 0.46±0.09 and 0.32±0.05 mg/dl, respectively. This was significantly (P<0.01) higher compared to the control group value of 0.26±0.04 mg/dl.

In group VII, group VIII, group IX and group X in female on day 28, the Bilirubin concentration were 0.29±0.05, 0.27±0.02, 0.36±0.09 and 0.36±0.06 mg/dl, respectively. Control group value was 0.28±0.05 mg/dl (Table 33 and Fig. 53 and 54).

Compared to control group other treated groups did not show any significant (P>0.05) variation in the bilirubin concentration value.

4.8.4.1.3 ALT

In group II, group III, group IV and group V in male on day 14, the serum ALT concentration were 51.68±0.83, 50.23±1.61, 52.93±1.49 and 49.83±1.75 IU/l, respectively. Control group value was 50.87±0.87 IU/l.

In group II, group III, group IV and group V in male on day 28, the serum ALT concentration were 49.20±1.83, 45.66±1.33, 50.23±2.08 and 53.34±0.99 IU/l, respectively. Control group value was 48.28±1.57 IU/l.

In group VII, group VIII, group IX and group X in female on day 14, the serum ALT concentration were 51.68±0.83, 51.90±0.80, 54.48±3.05 and 53.25±1.37 IU/l, respectively. Control group value was 50.87±0.87 IU/l.

In group VII, group VIII, group IX and group X in female on day 28, the serum ALT concentration were 52.20±1.33, 51.85±0.97, 56.07±1.99 and 61.68±3.19 IU/l, respectively. Control group value was 50.78±1.19 IU/l (Table.34 and Fig. 55 and 56).
Compared to control group other treated groups did not show any significant (P>0.05) variation in the ALT concentration value.

**4.8.4.1.4 AST**

In group II, group III, group IV and group V in male on day 14, the serum AST concentration were 48.45±2.96, 45.91±1.02, 50.21±1.33 and 53.91±2.26 IU/l respectively. Control group value was 49.21±1.47 IU/l.

In group II, group III, group IV and group V in male on day 28, the serum AST concentration were 45.19±1.39, 45.83±1.35, 48.75±2.34 and 54.20±1.29 IU/l respectively. Control group value was 53.45±0.72 IU/l.

In group VII, group VIII, group IX and group X in female on day 14, the serum AST concentration were 53.45±1.14, 61.00±2.35, 56.38±1.40 and 60.58±2.98 IU/l respectively. Control group value was 52.78±1.35 IU/l.

In group VII, group VIII, group IX and group X in female on day 28, the serum AST concentration were 53.45±2.93, 61.53±2.62, 56.38±1.40 and 60.58±2.98 IU/l respectively. Control group value was 56.70±2.98 IU/l (Table.35 and Fig. 57 and 58).

There was significant difference between control and treatment group of 0.1 ml at (P<0.05).

**4.8.4.1.5 Creatinine**

In group II, group III, group IV and group V in male on day 14 the corresponding serum creatinine concentration were 1.27±0.05, 1.15±0.03, 1.28±0.06 and 1.25±0.02 mg/dl, respectively. Control group values was 1.38±0.07 mg/dl.
In group II, group III, group IV and group V in male on day 28, the serum creatinine concentration were 1.26±0.04, 1.16±0.02, 1.24±0.03 and 1.29±0.02 mg/dl, respectively. Control value was 1.29±0.06 mg/dl.

In group VII, group VIII, group IX and group X in female on day 14 the corresponding serum creatinine concentration were 0.59±0.92, 0.29±0.04, 0.46±0.04 and 0.60±0.10 mg/dl, respectively. Control group values was 0.31±0.05 mg/dl.

In group VII, group VIII, group IX and group X in female on day 28, the serum creatinine concentration were 0.58±0.09, 0.26±0.05, 0.44±0.05 and 1.10±0.09 mg/dl, respectively. Control value was 0.37±0.02 mg/dl (Table 36 and Fig. 59 and 60).

There was no significant difference between control and treatment groups except in group IV and V on 14 and 28 days.

4.8.4.1.6 Total Serum Protein

In group II, group III, group IV and group V in male on day 14 the corresponding Total serum protein were 7.80±0.26, 7.45±0.10, 7.50±0.11 and 7.33±0.13 mg/dl. Control group values was 7.68±0.16 g/dl.

In group II, group III, group IV and group V in male on day 28, the total serum protein were 7.46±0.17, 7.26±0.13, 7.16±0.19 and 7.13±0.19 g/dl, respectively. Control group value was 7.58±0.23 g/dl.

In group VII, group VIII, group IX and group X in female on day 14 the corresponding total serum protein were 7.63±0.22, 7.11±0.27, 7.47±0.09 and 7.16±0.18 g/dl, respectively. Control group value was 7.51±0.20 g/dl.
In group VII, group VIII, group IX and group X in female on day 28, the total serum protein were 7.30±0.05, 7.26±0.13, 7.16±0.11 and 6.69±0.26 g/dl, respectively. Control group value was 7.31±0.24 g/dl (Table 37 and Fig. 61 and 62).

Compared to control group other treated groups did not show any significant variation (P>0.05) in the total serum protein concentration levels.

4.8.4.1.7 ALP

In group II, group III, group IV and group V in male on day 14, the serum ALP concentration were 133.18±1.51, 135.33±1.61, 130.06±2.78 and 124.1±2.45 IU/l, respectively. Control group value was 132.70±1.21 IU/l.

In group II, group III, group IV and group V in male on day 28, the serum ALP concentration were 141.25±3.93, 140.66±1.92, 133.80±1.22 and 132.20±2.84 IU/l, respectively. Control group value was 132.28±0.57 IU/l.

In group VII, group VIII, group IX and group X in female on day 14, the serum ALP concentration were 134.25±1.45, 135.33±1.61, 140.06±1.82 and 139.10±2.34 IU/l, respectively. Control group value was 132.70±1.20 IU/l.

In group VII, group VIII, group IX and group X in female on day 28, the serum ALP concentration were 137.92±1.71, 138.00±1.03, 140.36±2.51 and 152.20±1.45 IU/l, respectively. Control group value was 132.28±0.57 IU/l (Table 38 and Fig. 63 and 64).
4.8.5 Pathology

4.8.5.1 Gross pathology

There was no gross lesions noticed on completion of 28 day repeated oral safety study.

4.8.5.2 Histopathology

No histopathologic lesions noticed in rats in 28 day repeated oral safety study.

4.8.5.3 Sub-chronic toxicity study

4.8.5.3.1 Observation of clinical signs

Clinical signs were not evident in 0.05 ml, 0.1 ml and 0.2 ml dose groups. Dehydration, anorexia and slight reduction in body weight were found in high dose groups.

4.8.5.3.2 Body weight

The animals were weighed individually at the beginning of the study and at weekly intervals till the end of the study.

In group II, group III, group IV and group V in male on 30 day, the body weight were 195.50±3.89, 188.83±3.51 183.50±3.27 and 186.33±2.28 g, respectively. Control group value was 206.83±2.33 g.

In group II, group III, group IV and group V in male on 60 day, the body weight were 227.83±5.01, 225.17±3.66, 209.83±4.88 and 224.67±3.62 g, respectively. Control group value was 243.83±4.66g.
In group II, group III, group IV and group V in male on day 90, the body weight were 258.83±5.12, 261.50±2.58, 216.17±22.18 and 260.33±3.07g, respectively. Control group value was 276.00±5.53g.

In group VII, group VIII, group IX and group X in female on 30 day, the body weight were 180.67±2.32, 183.83±4.22, 181.00±2.83 and 182.83±2.93g, respectively. Control group value was 176.83±3.19g.

In group VII, group VIII, group IX and group X in female on 60 day, the body weight were 213.00±5.43, 216.67±4.25, 206.33±4.55 and 209.17±5.31 g respectively. Control group value was 209.83±6.63g.

In group VII, group VIII, group IX and group X in female on day 90, the body weight were 247.33±5.17, 216.67±4.25, 206.33±4.55 and 209.17±5.31g respectively. Control group value was 241.83±6.74 g (Table.39 and Fig. 65 and 66).

4.9 Hematology

4.9.1 Hematological parameters in male and female rats

4.9.1.1 TEC

In group II, group III, group IV and group V in male on 30 day the TEC were 7.91±0.24, 6.90±0.12, 6.80±0.17 and 7.21±0.29 x 10⁶/mm³ respectively. Control group value was 7.91±024 x10⁶/mm³. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml at (P<0.05).

In group II, group III, group IV and group V in male on 60 day the TEC were 7.56±0.27, 6.89±0.24, 6.51±0.11 and 6.90±0.23 x 10⁶/mm³ respectively. Control group value was 7.56±027 x 10⁶/mm³. There was no significant difference between control and treatment group of 0.2 ml at (P<0.05).
In group II, group III, group IV and group V in male on 90 day the TEC were 7.66±0.29, 7.10±0.28, 6.71±0.31 and 5.43±0.08 x 10^6/mm^3 respectively. Control group value was 7.66±0.29 x 10^6/mm^3. There was significant difference between control and treatment group of 0.2 ml at (P<0.05).

In group VII, group VIII, group IX and group X in female on 30 day the TEC were 7.37±0.28, 7.06±0.17, 7.15±0.23 and 7.04±0.14 x10^6/mm^3 respectively. Control group value was 7.90±027 10^6/mm^3.

In group VII, group VIII, group IX and group X in female on 60 day the TEC were 7.64±0.28, 6.74±0.20, 6.27±0.20 and 6.48±0.11 x10^6/mm^3 respectively. Control group value was 7.45±027 x10^6/mm^3. There was significant difference between control and treatment group of 0.2 ml at P<0.05.

In group VII, group VIII, group IX and group X in female on 90 day the TEC were 6.87±0.17, 6.61±0.20, 5.73±0.22 and 5.62±0.17 x10^6/mm^3 respectively. Control group value was 7.22±0.26 x 10^6/mm^3. There was significant difference between control and treatment groups of 0.2 ml, 0.3 ml at P<0.001 (Table 40 and Fig. 67 and 68).

4.9.1.2 Haematocrit

In group II, group III, group IV and group V in male on 30 day, the Haematocrit value were 42.29±1.13, 38.68±0.52, 34.23±1.31 and 34.18±1.82%, respectively. Control group value was 41.44±1.05%. There was significant difference between control and treatment group of 0.2 ml and 0.3ml at P<0.001.

In group II, group III, group IV and group V in male on 60 day, the haematocrit value were 39.64±0.91, 37.57±0.33, 30.88±1.19 and 29.58±0.34%, respectively. Control group value was 42.36±1.44 %.
In group II, group III, group IV and group V in male on 90 day, the haematocrit value were 39.71±0.88, 37.56±0.45, 29.97±1.33 and 28.60±1.67 %, respectively. Control group value was 43.27±1.33 %. There was significant difference between control and treatment group of 0.3 ml at (P<0.001).

In group VII, group VIII, group IX and group X in female on 30 day, the haematocrit value were 41.44±1.05, 39.34±1.08, 37.85±1.17 and 35.87±0.33%, respectively. Control group value was 39.11±0.38%.

In group VII, group VIII, group IX and group X in female on 60 day, the haematocrit value were 40.03±0.05, 36.05±0.78, 34.54±0.96 and 29.33±0.34 %, respectively. Control group value was 42.36±1.44%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml, 0.3 ml at (P<0.001).

In group VII, group VIII, group IX and group X in female on 90 day, the haematocrit value were 39.27±0.59, 34.47±0.80, 28.64±0.78 and 25.26±0.69 %, respectively. Control group value was 43.27±1.33% (Table 41 and Fig. 69 and 70).

4.9.1.3 Haemoglobin

In group II, group III, group IV and group V in male on 30 day, the corresponding Hb concentration were 15.16±0.34, 14.64±0.14, 14.17±0.31 and 13.25±0.32 g%, respectively. Control group value was 16.31±0.54 g%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml at (P<0.01, P<0.001).

In group II, group III, group IV and group V in male on 60 day, the corresponding Hb concentration were 14.76±0.30, 13.84±0.37, 12.86±0.44 and 11.05±0.17 g%, respectively. Control group value was 14.97±0.40 g%. There was
significant difference between control and treatment groups of 0.2 ml, 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the corresponding Hb concentration were 14.26±0.56, 12.45±0.28, 12.02±0.11 and 9.21±0.36 g%, respectively. Control group value was 15.68±0.36 g%. There was significant difference between control and treatment groups of 0.05 ml at P<0.05 and 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day, the corresponding Hb concentration were 14.14±0.48, 13.64±0.30, 13.00±0.44 and 13.70±0.41 g%, respectively. Control group value was 15.83±0.22 g%. There was no significant difference between control and 0.05 ml treatment group.

In group VII, group VIII, group IX and group X in female on 60 day, the corresponding Hb concentration were 14.31±0.34, 13.34±0.42, 11.92±0.50 and 10.38±0.25 g% respectively. Control group value was 15.46±0.29 g%. There was significant difference between control and treatment groups of 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml at P<0.01, P<0.001.

In group VII, group VIII, group IX and group X in female on 90 day, the corresponding Hb concentration were 14.34±0.33, 11.96±0.35, 10.52±0.20 and 8.71±0.38 g%, respectively. Control group value was 15.00±0.43 g%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.05 (Table 42 and Fig. 71 and 72).

4.9.1.4 MCV

In group II, group III, group IV and group V in male on 30 day, the MCV were 52.21±1.56, 53.02±2.30, 53.45±1.94 and 53.07±1.80 fl respectively. Control group value was 51.22±1.70 fl.
In group II, group III, group IV and group V in male on 60 day, the MCV were 56.15±1.90, 62.55±0.97, 64.57±0.86 and 61.79±1.86 fl respectively. Control group value was 53.22±0.94 fl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the MCV were 54.50±1.18, 64.62±1.07, 72.90±2.10 and 74.40±2.66 fl respectively. Control group value was 49.88±1.25 fl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at (P<0.001).

In group VII, group VIII, group IX and group X in female on 30 day, the MCV were 51.63±1.70, 53.02±2.30, 50.11±2.61 and 52.07±1.57 fl respectively. Control group value was 53.38±1.13 fl.

In group VII, group VIII, group IX and group X in female on 60th day, the MCV were 53.38±0.85, 62.55±0.97, 62.90±2.31 and 60.95±1.71 fl respectively. Control group value was 53.84±1.14 fl. There was significant difference between control and treatment group of 0.3 ml at (P<0.05).

In group VII, group VIII, group IX and group X in female on 90 day, the MCV were 54.38±1.48, 67.95±2.18, 73.23±3.15 and 74.41±2.66 fl, respectively. Control group value was 51.90±1.33 fl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001. (Table 43 and Fig. 73 and 74).

**4.9.1.5 MCH**

In group II, group III, group IV and group V in male on 30 day, the MCH were 19.93±0.52, 19.15±0.60, 18.84±0.26 and 18.67±0.32 pg, respectively. Control group value was 20.69±0.48 pg.
In group II, group III, group IV and group V in male on 60 day, the MCH were 20.50±0.65, 16.89±0.41, 17.69±0.36 and 17.78±0.52 pg, respectively. Control group value was 20.96±0.43 pg. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the MCH were 19.33±0.54, 15.68±0.53, 15.68±0.52 and 14.23±0.37 pg, respectively. Control group value was 20.25 ± 0.18 pg. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day, the MCH were 20.69±0.48, 19.15±0.60, 19.15±0.60 and 18.51±0.28 pg respectively. Control group value was 21.80±0.34 pg. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 60 day, the MCH were 20.63±0.39, 16.88±0.41, 16.88±0.41 and 17.52±0.35 pg respectively. Control group value was 22.44±0.54 pg. There was significant difference between control and treatment groups of 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml at P<0.01, P<0.001.

In group VII, group VIII, group IX and group X in female on 90 day, the MCH were 19.91±0.20, 15.62±0.50, 15.62±0.50 and 15.51±0.46 pg, respectively. Control group value was 21.99±0.47 pg (Table 44 and Fig. 75 and 76).

4.9.1.6 MCHC

In group II, group III, group IV and group V in male on 30 day, the MCHC were 40.62±0.61, 37.48±1.24, 39.40±1.74 and 38.80±1.76 g/dl respectively.
Control group value was 39.55±0.66 g/dl. There was no significant difference between control and treatment groups.

In group II, group III, group IV and group V in male on 60 day, the MCHC were 34.31±1.00, 36.68±0.98, 34.56±1.16 and 34.27±1.33 g/dl, respectively. Control group value was 35.47±0.72 g/dl. There was no significant difference between control and treatment groups.

In group II, group III, group IV and group V in male on 90 day, the MCHC were 36.36±1.95, 31.94±1.07, 30.15±1.38 and 29.34±1.14 g/dl respectively. Control group value was 35.21±2.03 g/dl. There was significant difference between control and treatment group of 0.3 ml at (P<0.01).

In group VII, group VIII, group IX and group X in female on 30 day, the MCHC were 39.55±0.66, 37.46±1.25, 37.53±0.70 and 38.79±1.75 g/dl, respectively. Control group value was 41.46±0.58 g/dl.

In group VII, group VIII, group IX and group X in female on 60 day, the MCHC were 35.31±0.76, 36.70±0.98, 34.58±1.10 and 33.93±1.30 g/dl respectively. Control group value was 38.81±0.55 g/dl. There was significant difference between control and 0.05 ml treatment group.

In group VII, group VIII, group IX and group X in female on 90 day, the MCHC were 32.17±0.64, 31.42±0.80, 30.14±1.38 and 29.41±1.13 g/dl, respectively. Control group value was 40.42±1.05 g/dl. There was significant difference between control and treatment groups of 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml at P<0.001 (Table 45 and Fig. 77 and 78).
4.9.1.7 Eosinophils

In group II, group III, group IV and group V in male on 30 day, the eosinophils were 0.98±0.06, 0.98±0.04, 1.08±0.08 and 1.20±0.01% respectively. Control group value was 0.96±0.06%. There was significant difference between control and treatment groups of 0.05 ml at P<0.05 and 0.2 ml at P<0.01.

In group II, group III, group IV and group V in male on 60 day, the eosinophils were 0.98±0.04, 0.94±0.05, 1.06±0.07 and 1.06±0.07%, respectively. Control group value was 1.06±0.11%. There was significant difference between control and treatment groups of 0.05 ml at P<0.05.

In group II, group III, group IV and group V in male on 90 day, the eosinophils were 1.04±0.04, 0.91±0.05, 0.71±0.12 and 0.71±0.12%, respectively. Control group value was 1.11±0.06%. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.01.

In group VII, group VIII, group IX and group X in female on 30 day, the eosinophils were 1.04±0.07, 0.98±0.04, 1.07±0.08 and 1.18±0.01%, respectively. Control group value was 1.11±0.04%. There was no significant difference between control and 0.05 ml treatment group.

In group VII, group VIII, group IX and group X in female on 60 day, the eosinophils were 1.06±0.11, 0.94±0.05, 1.06±0.07 and 1.07±0.06%, respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 1.14±0.06%.

In group VII, group VIII, group IX and group X in female on 90 day, the eosinophils were 1.12±0.06, 0.91±0.05, 0.71±0.12 and 0.74±0.12%, respectively. Control group value was 1.14±0.03%. There was significant difference between
control and treatment groups of 0.2 ml, 0.3 ml at P<0.001 (Table 46 and Fig.79 and 80).

4.9.1.8 Lymphocytes

In group II, group III, group IV and group V in male on 30 day, the lymphocytes were 80.70±0.69, 72.84±1.74, 73.51±1.62 and 72.85±1.63 % respectively. Control group value was 80.94±1.58 %. There was significant difference between control and treatment group of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 60 day, the lymphocytes were 79.95±0.88, 68.96±1.29, 66.96±1.72 and 65.76±1.40 %, respectively. Control group value was 79.33±0.51 %. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the lymphocytes were 74.93±1.84, 64.78±2.02, 59.00±2.12 and 58.46±2.03 %, respectively. Control group value was 79.46±0.80 %. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group VII, VIII, IX and X in female on 30 days the lymphocytes were 80.94±1.58, 72.51±1.54, 73.34±1.50 and 72.85±1.63%, respectively. Control group value was 80.89±1.15%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.01, P<0.001.

In group VII, VIII, IX and X in female on 60 days, the lymphocytes were 79.33±0.51, 66.96±1.23, 66.79±1.68 and 65.76±1.40%, respectively. Control
group value was 81.33±0.71%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 90 day, the lymphocytes were 77.62±0.45, 62.61±1.46, 59.02±2.13 and 58.34±2.13%, respectively. Control group value was 81.33±0.71%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001 (Table 47 and Fig. 81 and 82).

4.9.1.9 TLC

In group II, group III, group IV and group V in male on 30 day, the TLC were 8.91±0.33, 9.04±0.41, 9.54±0.19 and 9.06±0.24 x 10^3/mm^3, respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 9.18±0.40 %.

In group II, group III, group IV and group V in male on 60 day, the TLC were 9.34±0.26, 8.19±0.31, 7.90±0.27 and 7.67±0.23 x 10^3/mm^3, respectively. There was significant difference between control and 0.2 ml at P<0.05 and 0.3 ml at P<0.01 treatment groups. Control group value was 9.43±0.29 x 10^3/mm^3.

In group II, group III, group IV and group V in male on 90 day, the TLC were 8.52±0.30, 8.01±0.23, 6.43±0.21 and 6.46±0.15 x 10^3/mm^3, respectively. Control group value was 9.80±0.55 x 10^3/mm^3. There was significant difference between control and treatment groups of 0.1 ml, at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day the TLC were 9.18±0.40, 8.88±0.32, 9.44±0.15 and 9.06±0.24 x 10^3/mm^3, respectively. Control group value was 9.51±0.14 x 10^3/mm^3. There was no significant difference between control and treatment groups.
In group VII, group VIII, group IX and group X in female on 60 day, the TLC were 9.60±0.41, 8.19±0.31, 7.90±0.27 and 7.68±0.22 x 10^3/mm^3, respectively. Control group value was 10.27±0.33 x 10^3/mm^3. There was significant difference between control and treatment groups of 0.1 ml at P<0.05 and 0.2 ml, 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 90 day, the TLC were 8.73±0.34, 7.84±0.24, 6.43±0.21 and 6.47±0.14 x 10^3/mm^3, respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 9.84±0.29 x 10^3/mm^3. (Table 48 and Fig. 83 and 84).

4.9.1.10 Monocyte

In group II, group III, group IV and group V in male on 30 day, the monocyte were 1.13±0.07, 0.87±0.04, 1.26±0.09 and 1.44±0.12 %, respectively. There was significant difference between control and 0.05 ml treatment group. Control group value was 1.22±0.09 %.

In group II, group III, group IV and group V in male on 60 day, the monocyte were 1.15±0.07, 0.85±0.05, 1.08±0.10 and 1.48±0.06 %, respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 1.17±0.08 %.

In group II, group III, group IV and group V in male on 90 day, the monocyte were 1.12±0.12, 0.74±0.04, 0.82±0.05 and 0.82±0.05 %, respectively. Control group value was 1.26±0.15 %. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at (P<0.01, P<0.001).

In group VII, group VIII, group IX and group X in female on 30 day, the monocyte were 1.15±0.04, 0.94±0.09, 1.17±0.05 and 1.25±0.11%, respectively.
There was no significant difference between control and 0.05 ml treatment group. Control group value was 1.15±0.03 %.

In group VII, group VIII, group IX and group X in female on 60 day, the monocyte were 1.17±0.08, 0.85±0.05, 1.08±0.10 and 1.17±0.07 %, respectively. Control group value was 1.18±0.07%. There was significant difference between control and treatment groups of 0.1 ml at P<0.01.

In group VII, group VIII, group IX and group X in female on 90 day, the monocyte were 1.26±0.15, 0.74±0.04, 0.82±0.05 and 0.82±0.05 %, respectively. Control group value was 1.14±0.07%. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.05, P<0.001 (Table 49 and Fig. 85 and 86).

4.9.2 Neutrophils

In group II, group III, group IV and group V in male on 30 day, the neutrophil were 20.91±1.16, 18.72±0.57, 22.05±0.84 and 22.51±0.60 %, respectively. Control group value was 17.86±0.40 %. There was significant difference between control and treatment groups of 0.05 ml at P<0.05 and 0.2 ml and 0.3 ml at P<0.01, P<0.001.

In group II, group III, group IV and group V in male on 60 day, the neutrophil were 21.36±0.77, 24.22±1.54, 26.56±1.06 and 27.40±0.72 %, respectively. Control group value was 18.34±0.35 %. There was significant difference between control and treatment groups of 0.05 ml at P<0.05, 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the neutrophil were 21.51±0.61, 27.22±0.87, 29.77±0.81 and 32.50±0.77 %, respectively. Control group value was 19.44±0.56 %. There was significant
difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day, the neutrophil were 17.86±0.40, 18.72±0.57, 22.08±0.85 and 22.51±0.60 %, respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 20.86±0.87 %.

In group VII, group VIII, group IX and group X in female on 60 day, the neutrophil were 18.18±0.61, 27.22±0.87, 29.78±0.81 and 32.50±0.77 %, respectively. Control group value was 19.95±0.42 %. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

On day 90, there was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001. Control group value was 20.25±0.50 % (Table 50 and Fig. 87 and 88).

### 4.9.3 Biochemical parameters

#### 4.9.3.1 Male and female rats

##### 4.9.3.1.1 BUN

In group II, group III, group IV and group V in male on day 30, the BUN concentration was 24.95±0.74, 25.04±1.01, 27.40±0.48 and 27.50±0.78 mg/dl, respectively. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.01. Control group value was 22.98 ± 1.06 mg/dl.

In group II, group III, group IV and group V in male on day 60, the BUN concentration was 25.83±0.96, 24.83±0.56, 27.41±1.12 and 30.56±1.23 mg/dl,
respectively. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.01. Control group value was 2.93±0.74 mg/dl.

In group II, group III, group IV and group V in male on day 90, the BUN concentration was 25.46±0.83, 24.41±0.82, 27.95±0.56 mg/dl and 33.50±2.12 mg/dl in group III. Control group value was 22.44±0.80 mg/dl. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 30, the BUN concentration were 22.97±0.65, 27.88±0.48, 27.42±0.49 and 26.62±0.96 mg/dl, respectively. Control group value was 22.88 ± 1.09 mg/dl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.01.

In group VII, group VIII, group IX and group X in female on day 60, the BUN concentration were 24.62±1.11, 26.69±0.84, 29.07±1.09 and 38.41±1.29 mg/dl respectively. Control group value was 20.91±1.08 mg/dl. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 90, the BUN concentration were 23.23±0.71, 27.91±0.07, 27.97±0.57 mg/dl and 54.15±2.26 mg/dl, respectively. Control group value was 24.05±0.91 mg/dl. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.05 and P<0.001 (Table 51 and Fig. 89 and 90).

4.9.3.1.2 Bilirubin

In group II, group III, group IV and group V in male on day 30, the bilirubin concentration were 0.29±0.02, 0.35±0.02, 0.23±0.05 and 0.28±0.05
mg/dl, respectively. There was significant difference between control and 0.2 ml treatment group P<0.05. Control group value was 0.30 ± 0.05 mg/dl.

In group II, group III, group IV and group V in male on day 60, the bilirubin concentration were 0.36±0.02, 0.43±0.02, 0.22±0.04 and 0.31±0.03 mg/dl, respectively. There was significant difference between control and 0.05 ml treatment group at P<0.05 and 0.1 ml at P<0.01. Control group value was 0.27±0.01 mg/dl.

In group II, group III, group IV and group V in male on day 90, the bilirubin concentration were 0.26±0.04, 0.34±0.09, 0.28±0.04mg/dl and 0.68±0.22 mg/dl, respectively. Control group value was 0.20±0.05 mg/dl. There was significant difference between control and treatment groups of 0.1 ml at P<0.01, 0.2 ml at P<0.05 and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 30, the bilirubin concentration were 0.30±0.02, 0.36±0.02, 0.32±0.06 and 0.30±0.05 mg/dl, respectively. There was no significant difference between control and treatment groups. Control group value was 0.31±0.05 mg/dl.

In group VII, group VIII, group IX and group X in female on day 60, the bilirubin concentration were 0.33±0.02, 0.43±0.02, 0.29±0.07 and 0.81±0.04 mg/dl in group III. Control group value was 0.27±0.01 mg/dl. There was significant difference between control and treatment groups 0.05 ml and 0.1 ml at P<0.05 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 90, the bilirubin concentration were 0.26±0.06, 0.34±0.08, 0.28±0.04 mg/dl and 0.99±0.08 mg/dl. Control group value was 0.28±0.02 mg/dl. There was
significant difference between control and treatment groups of 0.3 ml at P<0.001 (Table 52 and Fig. 91 and 92).

4.9.3.1.3 ALT

In group II, group III, group IV and group V in male on 30 day the serum ALT concentration were 50.46±1.04, 50.66±2.04, 53.49±1.66 and 58.60±3.45 IU/l respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 51.22±0.94 IU/l.

In group II, group III, group IV and group V in male on 60 day, the serum ALT concentration were 51.82±1.21, 58.42±3.52, 55.77±1.00, and 64.31±3.23 IU/l, respectively. Control group value was 51.98±1.63 IU/l. There was significant difference between control and treatment group of 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the serum ALT concentration were 54.50±1.74, 59.12±3.77, 59.51±2.34 and 76.70±1.68 IU/l, respectively. Control group value was 51.58±2.04 IU/l. There was significant difference between control and treatment group of 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day the serum ALT concentration were 50.46±1.01, 47.83±1.86, 49.82±1.46 and 61.77±3.31 IU/l, respectively. Control group value was 51.24±0.93 IU/l. There was no significant difference between control and treatment groups.

In group VII, group VIII, group IX and group X in female on 60 day, the serum ALT concentration were 52.40±1.22, 45.43±1.10, 59.43±3.18, and 80.97±3.70 IU/l, respectively. There was significant difference between control and 0.3 ml treatment group at P<0.001. Control group value was 52.50±1.53 IU/l.
In group VII, group VIII, group IX and group X in female rats on 90 day, the serum ALT concentration were 51.00±2.44, 52.45±1.52, 59.84±3.12 and 99.56±3.54 IU/l, respectively. Control group value was 54.77±2.01 IU/l. There was significant difference between control and treatment group of 0.3 ml at P<0.001 (Table 53 and Fig. 93 and 94).

4.9.3.1.4 AST

In group II, group III, group IV and group V in male on 30 day the serum AST concentration were 59.52±2.55, 62.96±2.03, 57.36±2.14 and 68.04±3.43 IU/l, respectively. There was no significant difference between control and treatment groups. Control group value was 63.43±2.13 IU/l.

In group II, group III, group IV and group V in male on 60 day, the serum AST concentration were 59.83±2.29, 65.51±1.06, 63.83±1.38 and 79.14±4.02 IU/l, respectively. Control group value was 58.42±2.87 IU/l. There was significant difference between control and treatment group of 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the serum AST concentration were 55.69±1.12, 64.58±1.25, 66.29±1.98 IU/l and 92.66±4.72 IU/l, respectively. Control group value was 58.55±1.98 IU/l. There was significant difference between control and treatment group of 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day the serum AST concentration were 62.05±2.59, 62.96±1.82, 59.02±1.70 and 65.20±2.68 IU/l, respectively. There was no significant difference between control and treatment groups. Control group value was 61.79±2.02 IU/l.

In group VII, group VIII, group IX and group X in female on 60 day, the serum AST concentration were 61.32±1.67, 65.33±2.52, 58.35±1.94 and
84.48±2.37 IU/l, respectively. Control group value was 58.80±3.02 IU. There was significant difference control found between control and treatment groups of 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 90 day, the serum AST concentration were 61.19±3.04, 64.58±0.95, 65.32±1.04 and 104.73±4.20 IU/l, respectively. Control group value was 59.90±1.84 IU/l. There was significant difference between control and treatment groups of 0.3 ml at P<0.001 (Table 54 and Fig. 95 and 96).

4.9.3.1.5 TSP

In group II, group III, group IV and group V in male on 30 day the total serum protein concentration were 7.63±0.22, 7.13±0.22, 7.13±0.14 and 7.32±0.16 g/dl, respectively. There was significant difference between control and 0.05 ml treatment group. Control group value was 7.35±0.17 g/dl.

In group II, group III, group IV and group V in male on 60 day, the total serum protein concentration were 7.13±0.18, 6.97±0.20, 6.97±0.20 and 6.64±0.20 g/dl, respectively. Control group value was 7.32±0.14 g/dl. There was significant difference between control and treatment group of 0.2 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the total serum protein concentration were 7.13±0.16, 6.78±0.19, 6.38±0.18, and 6.07±0.25 g/dl respectively. Control group value was 7.40±0.18 g/dl. There was significant difference between control and treatment group of 0.2 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on 30 day the total serum protein concentration were 7.57±0.17, 7.05±0.24, 7.18±0.21 and 7.06±0.18 g/dl, respectively. There was no significant difference between control and treatment groups. Control group value was 7.50±0.47 g/dl.
In group VII, group VIII, group IX and group X in female on 60 day, the total serum protein concentration were 7.57±0.17, 7.00±0.27, 6.98±0.17 and 6.67±0.16 g/dl, respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 7.12±0.27 g/dl.

In group VII, group VIII, group IX and group X in female on 90 day, the total serum Protein (TSP) concentration were 7.38±0.08, 6.70±0.18, 6.73±0.10 and 5.88±0.28 g/dl respectively. Control group value was 7.92±0.26 g/dl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001 (Table 55 and Fig. 97 and 98).

4.9.3.1.6 ALP

In group II, group III, group IV and group V in male on 30 day the serum ALP concentration were 136.39±2.20, 138.12±1.57, 135.15±2.27 and 140.82±2.71 IU/l, respectively. There was no significant difference between control and treatment groups. Control group value was 132.70±1.21IU/l.

In group II, group III, group IV and group V in male on 60 day, the serum ALP concentration were 137.12±1.09, 141.57±3.03, 143.62±4.45 and 151.99±3.05 IU/l, respectively. Control group value was 132.28±0.57 IU/l. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.001.

In group II, group III, group IV and group V in male on 90 day, the serum ALP concentration were 133.97±2.81, 137.68±3.27, 147.33±3.01 and 157.80±4.96 IU/l, respectively. Control group value was 132.70±1.21 IU/l. There was significant difference between control and treatment groups of 0.2 ml and 0.3 ml at P<0.001.
In group VII, group VIII, group IX and group X in female on 30 day the serum ALP concentration were 137.64±2.44, 133.29±1.61, 135.49±2.11 and 141.34±3.26 IU/l, respectively. There was no significant difference between control and treatment 0.05 ml group. Control group value was 133.15±1.17 IU/l.

In group VII, group VIII, group IX and group X in female on 60 day, the serum ALP concentration were 141.12±3.32, 133.14±2.71, 140.82±1.57 and 153.48±4.72 IU/l, respectively. Control group value was 132.47±0.87 IU/l. There was significant difference between control and treatment group of 0.3 ml at (P<0.001).

In group VII, group VIII, group IX and group X in female on 90 day, the serum ALP concentration were 141.30±1.54, 133.62±3.70, 143.67±2.00 and 170.98±3.80 IU/l, respectively. Control group value was 133.90±1.20 IU/l. There was significant difference between control and treatment group of 0.3 ml at (P<0.001). (Table 56 and Fig. 101 and 102).

4.9.3.1.7 Creatinine

In group II, group III, group IV and group V in male on day 30, the serum creatinine concentration were 0.51±0.02, 0.68±0.11, 0.80±0.03 mg/dl and 0.95±0.05 mg/dl respectively. There was no significant difference between control and 0.05 ml treatment group. Control group value was 0.50±0.01 mg/dl.

In group II, group III, group IV and group V in male on day 60, the serum creatinine concentration were 0.52±0.04, 0.63±0.03, 0.72±0.05 mg/dl and 1.26±0.04 mg/dl respectively. Control group value was 0.52±0.04 mg/dl. There was significant difference between control and treatment group of 0.2 ml, 0.3 ml at P<0.001.
In group II, group III, group IV and group V in male on day 90, the serum creatinine concentration were 0.60±0.08, 1.02±0.05, 1.39±0.07 mg/dl and 1.55±0.04 respectively. Control group value was 0.59±0.08 mg/dl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 30, the serum creatinine concentration were 0.66±0.12, 0.68±0.11, 0.86±0.04 mg/dl and 0.46±0.06 g/dl respectively. Control group value was 0.48±0.03 mg/dl. There was significant difference between control and treatment group of 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 60, the serum creatinine concentration were 0.59±0.04, 0.66±0.12, 0.68±0.11 mg/dl and 0.86±0.04 mg/dl respectively. Control group value was 0.54±0.07 mg/dl. There was significant difference between control and treatment group of 0.3 ml at P<0.001.

In group VII, group VIII, group IX and group X in female on day 90, the serum creatinine concentration were 0.38±0.05, 1.10±0.07, 1.49±0.04 and 1.70±0.04 mg/dl respectively. Control group value was 0.54±0.07 mg/dl. There was significant difference between control and treatment groups of 0.1 ml, 0.2 ml and 0.3 ml at P<0.001 (Table 57 and Fig. 101 and 102).

4.9.4 Pathology

4.9.4.1 Gross pathology

Animals in cow urine treated groups did not show any gross pathological lesions in any of the organs in either of the sexes.
4.9.5 Histopathology

4.9.5.1 Histopathology of male and female rats in 0.3 ml dose

Organs retained normal appearance without any lesions.

4.9.5.2 Histopathology of male and female rats in 0.2 ml dose group

There was no change in gross as well as microscopic changes. Organs retained normal appearance with maintenance of normal architecture.

4.9.5.3 Histopathology of male and female rats 0.1 ml dose group

Organs from all the animals of these groups showed normal appearance with maintenance of normal architecture.

4.9.5.4 Histopathology of male and female rats in 0.05 ml dose group

Organs retained normal appearance without any lesions.

4.10 Physical, chemical and microscopic properties of cow urine in different cattle breeds:

4.10.1 pH

pH in Holstein Friesian cows was 8.20±0.06, Amruthmahal cows was 8.29±0.05, Jersy cows was 8.19±0.04, Saahiwal cows was 8.29±0.09, Hallikar cows was 8.28±0.05, Gir cows was 8.24±0.08, Malnad gidda cows was 8.19±0.11, Ongole cows was 8.33±0.09, Deoni cows was 8.24±0.11 and Kankrej cows was 8.27±0.09.

4.10.2 Specific gravity

Specific gravity in HF cows was 1.03±0.02, Amruthmahal cows was 1.04±0.30, Jersy cows was 1.02±0.00, Saahiwal cows was 1.04±0.40, Hallikar cows was 1.02±0.00, Gir cows was 1.02±0.00, Malnad gidda cows was 1.01±0.00,
Ongole cows was 1.01±0.00, Deoni cows was 1.01±0.00, Kankrej cows was 1.02±0.01 (Table 58 and Fig. 103).

There was no significant difference between breeds in pH and specific gravity value.

Urine samples in different cow breeds were found to be negative for microorganism, casts, crystals, blood cells, glucose, ketone bodies, bile salts and bile pigments.