V. DISCUSSION

The present study was carried out to assess the pharmacological activities and safety of cow urine in Wistar albino rats. The results obtained in respect of various parameters in various experiments under pharmacological activities viz., analgesic, antipyretic, anti-inflammatory, wound healing, hepatoprotective activity, immunomodulatory studies as well as acute toxicity, sub-acute toxicity and sub-chronic toxicity of urine from cross bred HF cows and also comparative study involving routine physical, chemical and microscopic parameters of urine from different breeds of cows are discussed here.

5.1 Evaluation of analgesic activity of cow urine

5.1.1 Male rats

Analgesic activity of cow urine was assessed in male rats, cow urine did not show analgesic activity. The reaction time in seconds in various treatment groups did not differ significantly at any interval when compared to control group I. But in group VI administered ibuprofen, the reaction times were significantly increased (P<0.01, P<0.001). There was no significant difference found between control and cow urine treated groups in male rats.

5.1.2 Female rats

Analgesic activity of cow urine was carried out in female rats, cow urine did not show analgesic property, reaction times in treatment group VII, VIII, IX, X and XI groups did not differ significantly when compared to control group. There was significant increase in reaction time in reference drug treated group (P<0.001).
Apparently there were no studies conducted earlier in cow urine for analgesic activity.

5.2 Evaluation of anti pyretic activity of cow urine

Anti pyretic activity of cow urine was assessed in male and female rats. There was significant decrease in experimentally induced pyrexia at different intervals in male and female rats and there was no significant difference found between control and urine treated groups II, III, IV and V. There are no studies conducted earlier on cow urine for antipyretic activity. Hence it’s difficult to interpret.

5.3 Evaluation of anti-inflammatory activity

The development of formalin-induced edema was biphasic; the first phase was attributed to the release of histamine, 5-HT and kinins occurred within an hour of injection and was partly due to the trauma of injection, while the second phase was related mainly to prostaglandins (Larsen and Henson, 1983; Vane and Booting, 1987) measured around 3 h.

Formalin-induced paw edema was considered one of the most suitable test procedures to screen chronic anti-inflammatory agents, as it closely resembles human arthritis (Greenwald, 1991). The nociceptive effect of formalin was also biphasic, an early neurogenic component followed by a tissue-mediated response (Wheeler-Aceto and Cowan, 1991).

NSAIDs inhibit cyclo-oxygenase in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors (Fields, 1987).
In the present anti-inflammatory study in rats, there was no significant decrease in paw thickness in cow urine treated groups when compared to control. There was significant decrease in paw thickness in diclofenac drug treated group (standard control) in both the sexes. The literature on anti-inflammatory activity of cow urine is scanty; hence no comparison could be made.

5.4 Evaluation of hepatoprotective activity of cow urine

Carbon tetrachloride is one of the most commonly used hepatotoxin. It was well documented that carbon tetrachloride is biotransformed under the action of cytochrome P450 in the microsomal compartment of liver to trichloromethyl radical which readily reacts with molecular oxygen to form trichloromethylperoxy radical (Raucy et al, 1993). Both the radicals can bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (Recnagel, 1983). This leads to the formation of lipid peroxides followed by pathological changes such as decreased the level of protein synthesis and elevated level of serum marker enzymes such as SGOT, SGPT and ALP and increase in lipid peroxidation (Recnagel, 1983).

The elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Thus lowering of enzyme content in serum is a definite indication of hepatoprotective action of a drug. SGPT catalyses the conversion of alanine to pyruvate and glutamate
and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage.

This toxic chemical caused peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane (Okuno et al., 1986).

Cow urine at the dose of 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml resulted in significant decrease in the levels of both AST and ALT.

Administration of cow urine showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. The effect was pronounced with all the treatment groups in both male and female rats. But the effect was not to the extent of silymarin treated animals.

The results are in agreement with the study of Achliya et al., (2003) where hepatoprotective effect of panchagavya was studied, and found that treatment with panchgaavya in doses (150-300 mg/ kg) significantly prevented the rise in the levels of serum marker enzymes viz., ALT, AST, ACP and ALP and the results obtained were comparable with those of silymarin treated group.
5.4.1 Pathology

5.4.1.1 Gross pathology

On autopsy, rats in group II of hepatoprotective activity showed generalized paleness of mucous membranes of all visceral organs. All other treated groups showed mild gross pathological lesions in liver in both the sexes.

5.4.1.2 Histopathology

Histopathology of liver showed loss of normal architecture with swollen hepatocytes and obliteration of sinusoidal spaces. Hepatocytes also showed granular and vacuolar appearance of cytoplasm (fatty change) in CCl4 treated group in both male and female rats. Histopathologic lesions in liver correlated with an increase in biochemical parameters AST, ALT and ALP levels.

In the groups treated with cow urine and the standard reference drug silymarin, histopathological sections of liver showed apparently normal architecture with mild congestion and vacuolar cytoplasm.

Further the levels are AST, ALT and ALP did not differ significantly between cow urine treated groups and the silymarin treated groups.

5.5 Wound healing property of cow urine

The wound healing efficacy of urine was evaluated in excision wound model. The parameter studied include rate of wound contraction.

The studies on excision wound healing revealed that all the groups showed decreased wound area from day to day. On day 4th the external application of urine showed significant increase in wound healing in male and
female rats compare to all other groups. However, on 14\textsuperscript{th} post wounding day, Group-I and VIII animals showed 0 \% and 0.4±0.4 \% of healing was left, which may be due to normal immunity of the animals. Where as nitrofurazone treated animals showed 0 \% and 0.5±0.3 \% healing was left. The significant increase in percent wound healing in external applied urine treatment could be correlated with the U S patent No. 6410059 for cow urine distillate, which claimed a novel pharmaceutical composition (Khanuja, 2002) and was effective as an antimicrobial and antifungal.

5.6 Evaluation of immunomodulatory study

The results obtained from parameters of humoral immune response and parameters of cell mediated immune response are discussed here.

5.6.1 Parameters of humoral immune response

The antibody titer, total serum immunoglobulin concentration, total serum protein concentration in male and female rats treated with cow urine did not show any significant variation (P>0.05) compared to control group. This indicated that cow urine did not exert any effect on humoral immune response.

5.6.2 Parameters of cell mediated immune response

Absolute lymphocyte count and DNBC skin sensitivity test in male and female rats treated with cow urine at 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml dose did not show any significant variation (P>0.05) compared to control group. This indicated that cow urine did not exert any effect on cell mediated immune response.
On the contrary, Chauhan et al., (2001) studied the immunomodulatory effect of cow urine in mice and found that cow urine enhances both T- and B-cell blastogenesis and also increased level of IgG. Kumar (2001) and Chauhan et al., (2004) reported increase in both cellular and humoral immune responses due to cow urine.

However in the current study no immunopotentiation in terms of humoral and cell mediated effects were observed. This could be due to variation in breed, age, feed and grazing patterns.

5.7 Acute toxicity study

Cow urine even at the highest dose (5ml/kg) tested did not result in mortality in both male and female rats indicating that cow urine is practically non toxic, further LD$_{50}$ value for cow urine could not be established.

5.8 Sub acute toxicity study

In the assessment and evaluation of the toxic characteristics of cow urine, this study provides information on the health effects and hazards likely to arise from repeated daily consumption over a relatively limited period of time. There were no clinical signs noticed in other groups.

5.8.1 Body weight

There was no significant difference between control and urine treated groups.

5.8.2 Hematological parameters
Hematological parameters were estimated using blood samples collected from all the animals on day 0, 14, 28. The hematological parameters viz., total erythrocyte count, hemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leucocyte count and differential leucocyte count were determined following standard methods. The present study, on day 28 there was significant (P<0.05) changes in hematological parameters of both male and female rats compared with the respective control group.

On day 28, there was significant (P<0.01) decrease in the total erythrocyte count \(10^6/mm^3\) and lymphocyte count (%) in the high dose group of both male and female rats compared with the respective control group.

On day 28, there was significant (P<0.01) increase in the neutrophil count in the high dose group of both male and female rats compared with the respective control group.

On day 28, there was significant (P<0.01) increase in MCV in the high dose group of both male and female rats compared with the respective control group.

On day 28, there was significant (P<0.01) decrease in MCHC in the high dose group of both male and female rats compared with the respective control group.

The observed increase in hematological parameters were within normal ranges of a healthy rat. Since there were no changes any change in clinical as well as histopathologic parameters and as the values were also within the normal range, the cow urine used is considered safe for daily consumption at the doses studied.
5.8.3 Biochemical parameters

Serum obtained from blood samples collected on day 0, 14, 28 of experiment period were used to estimate ALT, AST, ALP, creatinine, BUN, bilirubin and TSP for both male and female rats separately.

In the present study on day 14 and 28, the ALT values in high dose and intermediate dose group of male rats were significantly (P<0.01) higher than the respective control group.

On day 14, the AST values in high dose and intermediate dose group of female rats were significantly (P<0.05) higher than the control group.

On day 28, the AST values in high dose and intermediate dose group of female rats were significantly (P<0.05) higher than the control group.

On day 28, the ALT values in high dose group of both male and female rats were significantly (P<0.01) higher than the respective control group.

On day 14, the BUN concentrations in high dose and intermediate dose group of both male and female rats were significantly (P<0.01) higher than the respective control group.

On day 28, the BUN concentrations in high dose and intermediate dose group of both male and female rats were significantly (P<0.05) higher than the respective control group.

On day 14, the serum creatinine concentrations in high dose group of male rats were significantly (P<0.05) higher than the control group.
On day 28, the serum creatinine concentrations in high dose and intermediate dose group of male rats were significantly (P<0.05) higher than the respective control group.

On day 28, the serum creatinine concentrations in high dose group of female rats were significantly (P<0.01) higher than the respective control group.

The observed increase in biochemical parameters were between normal ranges of a healthy rat. Since we did not find any change in clinical as well as histopathologic differences, the values were also under the normal range the cow urine at the said dose groups said to be safe.

5.8.4 Pathology

5.8.4.1 Gross pathology

All organs were having normal architecture. No gross lesions were found in any of the treated groups.

5.8.4.2 Histopathology

Microscopically there was no lesions in any of the organs.

5.9 Sub chronic toxicity study

5.9.1 Body weight

There was significant difference between control and urine treated groups.

5.9.2 Hematological parameters

There was significant difference between control and urine treated groups. Though there was significant difference but these significant values are coming under normal range.
5.9.3 Biochemical parameters

There was significant difference between control and urine treated groups. Though there was significant difference but these significant values are coming under normal range.

5.10 Pathology

5.10.1 Gross pathology

There were no gross lesions found in any of the organs.

5.10.2 Histopathology

Microscopically no lesions were observed in any of the organs.

The various parameters revealed that cow urine is safe for consumption at the dose employed.

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

5.11.1 pH

There was no significant difference between breeds in pH value

5.11.2 Specific gravity

There was no significant difference between breeds in specific gravity values.

5.11.3 Microscopic properties
In all the breeds of the cattle the colour of urine was found to be yellow, appearance was clear. The urine was negative for the presence of proteins, ketone bodies, sugar, bile salts, bile pigments, casts, crystals and micro-organisms. Urobilin was found to be normal.

There were no significant differences in the breeds studied in terms of physical, chemical and microscopic properties of the cow urine.

The following conclusions were drawn from the present study.

1. Cow urine at the used dosage did not show analgesic, antipyretic, anti-inflammatory and immunomodulatory activity.

2. Cow urine at the used dosage produced hepatoprotective action.

3. Cow urine on external application enhanced wound healing activity.

4. Cow urine used in the present study did not result in death in both male and female rats even at the highest dose tested indicating that cow urine was safe.

5. Cow urine was also found to be safe on daily administration for 28 days and 90 days at the dose employed.

6. The mean pH of different breeds was found to be 8.19 - 8.33. The average specific gravity was found to be 1.01-1.04. There were no significant differences in various physical, chemical and microscopic properties of urine in different breeds studied.