EVALUATION OF ACUTE AND SUBCHRONIC ANTI-INFLAMMATORY EFFECT OF COW URINE IN RATS

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ABSTRACT

The study was conducted to know the anti-inflammatory effect of cow urine on formalin induced (edema Cacute and chronic) inflammatory activity in male and female Wistar albino rats procured from the Laboratory Animal Facility of Veterinary College, Hebbal, Bangalore. They were aged between six to seven weeks and weighed 130-150g. In the present study, there was no significant decrease in paw thickness in cow urine treated groups when compared to standard control. There was significant decrease in paw thickness in Diclofenac drug treated group (standard control) in both the sexes of rats. The literature on anti-inflammatory activity of cow urine is scanty; hence no comparison could be made.

Key words : Anti-inflammatory activity, Cow urine, Rats.

INTRODUCTION

Cattle husbandry was well developed during the Rigvedic period (1500–1000 BC) and the cow (Kamadhenu) was adored and considered the ‘best wealth’ of mankind. Atharvanaveda provides interesting information about ailments of animals, herbal medicines and cure of diseases. Urine of cows was considered as an antidote to poisons (Sushrut Samhita). Cows were regarded as wealth and were the backbone of the economy of ancient Indians. Cattle were one of the most frequently used animals described in the Vedas.

From the ancient period, cow’s urine has been used as a medicine. In Sushrut Samhita, several medicinal properties of cow’s urine have been mentioned and cow urine was known to cause weight loss and to cure leprosy, cardiac and kidney problems, indigestion, stomach ache, edema, etc. (Kaviratna and Sharma., 1996). “Kamadugha Yojane” has been drawn up to protect Indian cows in appreciation of the multifarious uses of “Panchagavya,” which comprises cow dung, urine, ghee, curd and milk which is found to be effective in treating major diseases such as cancer and diabetes. The fact that cow urine costs more than milk speaks of its limitless medicinal use (Raghaveshwara Bharati Swamiji, 2006).

United States Patent and Trade mark Office had granted Patents No 6410059 and No. 6896907 to an “Indian innovation which has proved that cow urine can make antibiotics, anti-fungal agents and also anti-cancer drugs more effective”. Other studies also revealed the immune-modulatory properties of cow urine. Besides, cow urine is said to be a very

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effective insect repellent when mixed with certain herbs (Nair, 2002). The re-distillate of cow’s urine was found to possess total antioxidant status of around 2.6 m mol, contributed mainly by volatile fatty acids (1500 mg/L) as revealed by the GC-MS studies. These fatty acids and other antioxidants might cause the observed protective effects (Krishnamurthi et al., 2004). The present research investigates the pharmacological activities and safety evaluation of freshly voided cow urine with the aim of providing scientific basis for use of cow urine in various disease conditions.

Hence, the present study was undertaken in male and female Wistar albino rats with the objective to study its anti-inflammatory activity.

**MATERIALS AND METHODS**

**Selection of experimental animals:** Wistar albino rats procured from the Laboratory Animal Facility of Veterinary College, Hebbal, Bangalore, were used in the present study. They were aged between six to seven weeks and weighed 130-150g. They were acclimatized to the experimental conditions for one week. After acclimatization, animals were grouped and housed in standard polypropylene rat cages during the experiment. They were maintained under standard laboratory hygienic conditions, providing standard laboratory animal feed and water ad libitum. The approval of the Institutional Animal Ethics Committee was obtained prior to start of the experiment.

**Selection of urine source:** Holstein-Friesian (HF) cross-bred, milking cows aged about 2-3 years were selected for urine collection. Animals were hygienically maintained in Karnataka Veterinary, Animal and Fisheries Sciences University regional campus, Department of Livestock Production and Management, Veterinary College, Hebbal, Bangalore. All the cows were fed with the concentrate prepared at the dairy farm and were provided clean drinking water ad libitum and daily animals were also taken outside for grazing in the university grazing land. All the animals were monitored carefully throughout the experimental period.

**Collection of urine:** Natural voiding mid-stream urine was collected in a sterile glass container. Collected urine was stored in refrigerator at 4°C and utilized for the experiment within two hours of collection. Precaution was taken to avoid any external contamination. Urine was collected from the same cows and used in experiment. Immediately after collection physical, chemical and microscopic examinations were done. Urine specimens were collected and stored as part of standard protocols in cohort studies designed to assess exposure to non persistent environmental contaminants (Gunter, 1997; Landi and Caporaso, 1997).

**Selection of doses for pharmacological assays:** The doses corresponding to the dose of 15 ml, 30 ml, 60 ml and 75 ml per day in a human being weighing around 60 kg. The doses administered were 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml/100 gm bodyweight in rats (The higher range of rats body weight was considered for dosage calculation).

**Administration of doses:** The fresh urine at 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml/100 gm was selected and was made up to 2 ml by adding distilled water and was gavaged using a stomach tube 1 hour before formalin injection and oedema observed for a period of 6 days in sub-chronic oral safety study.

**Experimental design :**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Male</th>
<th>Dose</th>
<th>Female</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Control</td>
<td>VII</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Diclofenac</td>
<td>VIII</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>0.05 ml</td>
<td>IX</td>
<td>6</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>0.1 ml</td>
<td>X</td>
<td>6</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>0.2 ml</td>
<td>XI</td>
<td>6</td>
</tr>
<tr>
<td>VI</td>
<td>6</td>
<td>0.3 ml</td>
<td>XII</td>
<td>6</td>
</tr>
</tbody>
</table>
Formalin-induced edema in rat hind paw

Oedema was induced by injecting 0.1 ml of 2% (w/v) formalin into the sub plantar region of the right hind paw of the rats according to the method described by Chau (1989). The rats of test groups (III-VI and IX-XII) were treated orally with 0.05 ml, 0.1 ml, 0.2 ml and 0.3 ml/100 gm body weight cow urine respectively, 1 h before formalin injection. The group (I) was control and the reference group (II and VIII) received Diclofenac at the dose of 10 mg/kg body weight (Diclofam® MAX) orally.

Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and the length of the thread corresponding to the paw circumference was determined using a meter ruler (Hess and Milonig, 1972; Olajide et al., 2000). Measurement was done immediately before and 3 h following formalin injections.

The percentage thickness was calculated according to the following formula (Olajide et al., 2000).

\[
\frac{(Ct - Co) control - (Ct - Co) treated}{(Ct - Co) control} \times 100
\]

Where \( Ct \) was the paw circumference at time \( t \), \( Co \) was the paw circumference before formalin injection, \( Ct-Co \) was oedema, \( (Ct - Co) control \) was oedema or paw size after formalin injection to control rats at time \( t \).

Evaluation of sub chronic anti-inflammatory effect of cow urine

Same procedure was adopted as mentioned in acute anti-inflammatory activity to evaluate the chronic anti-inflammatory activity of cow urine. But oedema was observed up to six days.

RESULTS AND DISCUSSION

Anti-inflammatory activity

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 1). There was no significant (\( P<0.05 \)) decrease in the inflammation in urine treated groups.

Sub 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 significant decrease in paw thickness in standard control group at \( P<0.01 \) (Table 2).

Acute anti-inflammatory activity in female rats:
The percentage thickness in the right paw in

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.05 ml</td>
<td>0.1 ml</td>
<td>0.2 ml</td>
<td>0.3 ml</td>
<td>Diclofenac</td>
</tr>
<tr>
<td>Male (%)</td>
<td>180.75±0.75</td>
<td>134.88±10.44</td>
<td>176.27±6.5</td>
<td>165.95±7.6</td>
<td>167.42±11.63</td>
<td>177.01±10.07</td>
</tr>
<tr>
<td>Female (%)</td>
<td>172.96±3.84</td>
<td>189.379.39</td>
<td>179.93±7.5</td>
<td>167.95±8.56</td>
<td>170.90±4.73</td>
<td>155.03±9.14</td>
</tr>
</tbody>
</table>

Values are mean ± SE, \( n = 6, P>0.05. \)

Table 1 : Effect of cow urine on acute anti-inflammatory activity in male and female rats.
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 1). There was no significant (P<0.05) decrease in the inflammation in treatment groups.

Sub 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 significant difference decrease in paw thickness in Diclofenac treated group at P<0.01 (Table 2).

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.

CONCLUSION

In the pharmacological studies, cow urine samples from HF cross bred cows were screened for anti-inflammatory activity in both sexes of Wistar Albino rats. The cow urine treated groups did not reveal any significant anti-inflammatory activity.

REFERENCES

Graph Pad Prism, Version 4.01 for Windows, Graph Pad Software Inc., (2004). San Diego, California, USA.


