INTRODUCTION

The species belonging Rungia have been claimed to exhibit antipyretic, diuretic, and antifungal properties. The whole plant of R. repens has been used throughout the ages for the treatment of fever and cough, and is also considered as vermifuge. Fresh, bruised leaves mixed with castor oil applied to scalp to cure tinea capitis, a scaly fungoid infection, usually occurring among children etc 1-3. Juice of the small and somewhat fleshy leaves of R. pectinata another Rungia species is considered cooling and aperient and is prescribed for children suffering from small pox in dose of a tablespoonful or two twice daily. The bruised leaves are applied to contusions to relieve pain and diminish swelling. Among the Santals, the root is given as a medicine in fevers 4.

MATERIALS AND METHODS

Plant Material

Rungia repens and Rungia pectinata plants were collected from nearby areas of Salipur, Orissa and identified at Botanical Survey of India, Howrah. Their voucher specimen was deposited in the Institute herbarium.

Preparation of Extracts

The shade dried aerial parts of R. repens and R. pectinata were powdered in a pulverizer separately. The powdered drug was extracted with Ethanol water mixture (50:50) for 72 h in Soxhlet apparatus. The concentrated extracts were transferred to preweighed flasks. After evaporation of the residual solvents the weight of each extract was recorded. The yields were 8.52 and 7.64 % w/w for R. repens and R. pectinata respectively. Phytochemical screening gave positive tests for carbohydrates, amino acids, fixed oils, phytosterols, glycosides, tannins and phenolic compounds. Interestingly both the plant contains similar type of phytoconstituents when subjected to preliminary phytochemical screening.

Experimental Animals

Wistar rats of either sex weighing 150-200 g were used for screening of anti-pyretic activity. Swiss albino mice of either sex weighing 20-25 g were used for screening analgesic activity. Animals were housed in groups of six per cage at a temperature of 25 ± 1°C and relative humidity of 70 ± 5%. A 12:12 hour light-dark cycle was followed during the experiments. Animals had free access to food and water, however, food was withdrawn six hours before and during the experiments. The animals were obtained from the Central Animal House of Institute of Pharmacy & Technology, Salipur. The experimental protocol was approved by the Institutional Ethical Committee (1053/ac/07/CPCSEA). The hydroalcoholic extract of both the plants were devoid of any mortality or behavioral changes when the animals were given upto 800 mg/kg p.o. in rats and mice.

Acetic acid induced writhing test

The hydroalcoholic extracts were evaluated for its analgesic activity by acetic acid-induced writhing model5, 7. Swiss albino mice were divided into six groups of six animals each. First group was used as negative control and received distilled water (5 ml/kg body weight), an hour before injection of 0.6 % v/v acetic acid (10 ml/kg) intraperitonially.

Second group served as positive control and received aspirin 200 mg/kg body weight p.o. The third and fourth group received the extracts of R. repens at dose levels of 400 mg/ kg and 800 mg/kg body weight respectively. Similarly fifth and sixth group received the extracts of R. pectinata at the same dose level an hour before acetic...
acid injection. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted for 15 minutes after administration of acetic acid. Percent protection against writhing movement was taken as index of analgesia.

Table 1: Effect of R. repens and R. pectinata on yeast induced pyrexia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Yeast Induced Pyrexia Temperature in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>37.71±0.12</td>
<td>37.68±0.14</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>150</td>
<td>37.16±0.09</td>
</tr>
<tr>
<td>R. repens</td>
<td>400</td>
<td>37.26±0.15</td>
</tr>
<tr>
<td>R. pectinata</td>
<td>400</td>
<td>37.65±0.07</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>37.14±0.09</td>
</tr>
<tr>
<td>Distilled H2O</td>
<td>1ml/kg</td>
<td>36.72±0.12</td>
</tr>
</tbody>
</table>

n=6, Values are mean ± SEM **P<0.01 (significant)** *P<0.05 (significant) values are compared with control group

Table 3: Effect of R. repens and R. pectinata on tail immersion response in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug dose mg/kg,i.p.</th>
<th>Predrug (mean±SEM) reaction time (in sec)</th>
<th>Reaction time in sec (mean + SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H2O</td>
<td>1ml/kg</td>
<td>3.5±0.4</td>
<td>4.3±0.49</td>
</tr>
<tr>
<td>R. repens</td>
<td>400</td>
<td>3.2±0.6</td>
<td>6.1±0.6</td>
</tr>
<tr>
<td>R. pectinata</td>
<td>400</td>
<td>3.0±0.5</td>
<td>8.0±0.73*</td>
</tr>
<tr>
<td>R. pectinata</td>
<td>800</td>
<td>2.7±0.3</td>
<td>5.0±0.51</td>
</tr>
<tr>
<td>Pethidine</td>
<td>5</td>
<td>2.8±0.4</td>
<td>5.3±0.61</td>
</tr>
<tr>
<td>Pethidine</td>
<td>800</td>
<td>3.7±0.3</td>
<td>9.0±0.73*</td>
</tr>
</tbody>
</table>

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet’s test. P <0.001 vs Control (n=6)

Tail immersion method
The method described by Siegmund et al. was used. Swiss albino mice were screened by exposure to the thermal stimulus. The mice showing positive response were divided into six groups of six animals each. The animals of first group was treated with distilled water and served as control. The group treated with pethidine (5 mg/kg, p.o) served as positive control. The third and fourth group received the extract of R. repens at dose levels of 400 mg/kg and 800 mg/kg body weight respectively. Similarly fifth and sixth group received the extract of R. pectinata at the same dose level. About 5 cm of the tail of mice was dipped in warm water, kept constant at 55 ± 0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 15 sec. The latent period of the tail immersion response was taken as the index on analgesic and was determined immediately after injection.

Statistical analysis
The statistical analysis was carried out with SPSS 10.0 (Windows) software. Difference of the parametric data of body temperature(s) was examined by two way analysis of variance (ANOVA) with Dunett’s Post hoc pairwise multiple comparison test, to compare a set of experimental data against control mean.

RESULTS
The average antipyresis recorded after 5 h of treatment with hydroalcoholic extract of R. repens were 0.89°C and 1.55°C at the dose levels of 400 and 800 mg/kg body weight respectively. Similarly, antipyresis for R. pectinata was also recorded as 1.3°C and 1.7°C at 400 and 800 mg/kg body weight dose levels respectively. The standard drug paracetamol at 150 mg/kg, p.o. body weight produced significant decrease in elevated body temperature. This study suggests that antipyresis produced by both the plants’ extract is directly proportional to the administered dose (Table 1 & Fig. 1). The results were found to be highly significant (P<0.05) in comparison to the control. The hydroalcoholic extracts of R. repens and R. pectinata (400 and 800 mg/kg, p.o) significantly suppressed the acetic acid-induced writhings in a dose-dependent manner. The standard drug aspirin at 200 mg/kg body weight produced significant inhibition of writhing movements (Table 2 & Fig. 2). The results were found to be highly significant (P<0.001) in comparison to the control. The number of writhing movements during 30 min of observation in the control group was 80.17±2.48 which corresponds with the findings of other workers11.12.

Fig. 1: Anti-pyretic activity of R. repens and R. pectinata
The hydroalcoholic extracts of R. repens and R. pectinata (400 and 800 mg/kg, p.o) significantly suppressed the acetic acid-induced writhings in a dose-dependent manner. The standard drug aspirin at 200 mg/kg body weight produced significant inhibition of writhing
The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet’s test. P <0.001 vs Control (n= 6).

Table 2: Effect of R. repens and R. pectinata on acetic acid induced writhing response in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Acetic acid induced writhing response in mice</th>
<th>No. of writhing movements</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>8ml/kg</td>
<td>80.17±2.48</td>
<td>-</td>
</tr>
<tr>
<td>R. repens 400</td>
<td></td>
<td>38.03±1.77*</td>
<td>51.56</td>
</tr>
<tr>
<td>R. repens 800</td>
<td></td>
<td>27.33±1.02*</td>
<td>65.91</td>
</tr>
<tr>
<td>R. pectinata 400</td>
<td></td>
<td>50.50±0.99*</td>
<td>37.01</td>
</tr>
<tr>
<td>R. pectinata 800</td>
<td></td>
<td>43.00±1.78*</td>
<td>46.36</td>
</tr>
<tr>
<td>Aspirin 100</td>
<td></td>
<td>16.67±0.88*</td>
<td>79.21</td>
</tr>
</tbody>
</table>

The results were found to be highly significant (P<0.001) in comparison to the control. The number of writhing movements during 30 min of observation in the control group was 80.17±2.48 which corresponds with the findings of other workers10, 11.

Moreover, the statistical analysis with two-way ANOVA showed that the hydroalcoholic extract of R. repens and R. pectinata decreases yeast induced elevated body temperature, thermal and acetic acid induced algies model in a dose dependent manner as compared with control group. The results were found to be highly significant (P<0.001) in comparison to the control.

DISCUSSION

Fever may be due to infection or one of the sequels of tissue damage, inflammation, graft rejection, or other disease states. Anti-pyretic are agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained.

In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature12. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature13. Elevated body temperature and pain are two major signs of the body against inflammation14.

A drug with anti-inflammatory activity usually exhibit anti-pyretic and analgesic properties15. The best examples would be the nonsteroidal anti-inflammatory drugs, which possess all three activities16. Previously, it was reported that R. repens and R. pectinata extract has diuretic, anti-inflammatory activity in rats and antimicrobial properties against a number of pathogenic microorganisms17.

The writhing response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is considered to involve local peritoneal receptors. The number of writhing movements during 30 min of observation in the control group was 80.17±2.48 which corresponds with the findings of other workers10, 11.

In the tail immersion model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center.

In the tail immersion model, there was no significant difference in the mean predrug reaction time between the different groups. Thirty minutes after drug administration, reaction time increased significantly for the test and standard groups when compared to the predrug reaction time. The test drug produced a dose-dependent increase in the reaction time at various time intervals of observation.

The mechanism underlying the activity of R. repens and R. pectinata in anti-pyretic and analgesic is still unknown. Preliminary phytochemical screening of the hydroalcoholic extract of both the plant species gave positive test for carbohydrates, amino acids, fixed oils, phytosterols, glycosides, tannins and phenolic compounds, which might be partly responsible for the anti-pyretic and analgesic activity reported in the current investigation.

Therefore, the overall results obtained suggested that the hydroalcoholic extract of R. repens and R. pectinata might relieve pain, provide some justification for the folklore use in the treatment of fever and pain. Further studies are going on to isolate the bioactive principles responsible for anti-pyretic and analgesic activities with their mechanism of action.

CONCLUSION

The hydroalcoholic extracts of R. repens and R. pectinata ariel part exhibits anti-pyretic and analgesic activity at a dose level of 400 and 800 mg/kg body weight. In comparison antipyretic activity of R.
pectinata is better than R. repens at the same dose level. Whereas, the hydroalcoholic extract of R. repens shown better analgesic activity as compared to R. pectinata. This finding provides some scientific evidence on the traditional use of both plants.

ACKNOWLEDGEMENT

The authors are grateful to the director and Department of Pharmacology, Institute of Pharmacy & Technology, Salipur for providing necessary facilities and financial help during the investigation.

REFERENCES