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Research Article

Efficacy of *Cynodon dactylon* for immunomodulatory activity

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MATERIALS AND METHODS

Plant material

Fresh leaves of *Cynodon dactylon* were collected in an area free of pesticides and other contaminants from the surrounding...
Preparation of proteins

Using Phosphate Buffered Saline (PBS), 20% extract of *Cynodon dactylon* fresh leaves were prepared and centrifuged at 5000 rpm for 10 minutes. The supernatant was subjected to the ammonium sulphate fractionation using 10-100% saturation of ammonium sulphate and the precipitates were dissolved in a known amount of PBS. Dialysis was done to desalt the *Cynodon dactylon* protein fractions (Cdpf).

Animals

Swiss albino mice weighing 25-30g of either sex were used in this study. They were procured from Perundurai Medical College, Perundurai. The animals were acclimatized for 15 days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C. They were fed with standard mice feed and water. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee prior to the beginning of the project work (889/ac/05/CPCSEA).

Antigen

SRBC collected in alsevers solution, washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5x10^6 cells mL^-1 for immunization and challenge.

Treatment

The animals were divided into three groups with 6 mice in each.

- **Group 1**: SRBC induced mice received pyrogallol (50mg/g body weight) in 100µl of PBS from 2nd to 14th day (i.p).
- **Group 2**: SRBC induced mice received 100µl of PBS from 2nd to 14th day (i.p).
- **Group 3**: SRBC induced mice received Cdpf 52µg in 100µl PBS from 2nd to 14th day (i.p).

Neutrophil Adhesion Test

On the 14th day of the Cdpf treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus onto heparinised vilas and analysed for Total Leukocyte Counts (TLC) and Differential Leukocyte Counts (DLC) by fixing blood smears and staining with field stain 1 and leishmans stain. After initial counts, blood samples were incubated with 80 mg mL^-1 of nylon fibers for 15 min at 38°C. The incubated blood samples were analyzed for TLC and DLC. The product of TLC and % neutrophil gives Neutrophil Index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below:

\[
\text{Neutrophil adhesion} \% = \left( \frac{\text{NI}_T - \text{NI}_U}{\text{NI}_U} \right) \times 100
\]

Where, \( \text{NI}_U \) = Neutrophil index of untreated blood sample; \( \text{NI}_T \) = Neutrophil index of treated blood sample

Haemagglutinating Antibody (HA) Titre

Mice of group III were pretreated with Cdpf for 14 days and each mouse was immunized with 0.5x10^9 SRBC/mice by i.p. route, including control mice. The day of immunization was referred as day 0. The animals were treated with Cdpf for 14 more days and blood samples were collected from each mice on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC. The micro titre plates were incubated at room temperature for two hours and examined visually for agglutination. The reciprocal of the highest dilution of serum showing 50% agglutination has been expressed as HA titre.

Delayed Type Hypersensitivity (DTH) Response

Six animals per group (control treated) were immunized on day 0 by i.p. administration of 0.5x10^9 SRBC/mice and challenged by a subcutaneous administration of 0.025x 10^9 SRBC/mL into right hind foot pad on day +14. Cdpf was administered orally from day +14 until day +13. The DTH response was measured at 24 h after SRBC challenge on +14 day and expressed as mean percent increase in paw volume.

Statistical Analysis

The data were analyzed using one-Way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparisons test. p<0.05 were considered significant.

RESULTS

*Cynodon dactylon* was evaluated for immunomodulatory effect. Cdpf showed a significant increase in neutrophil adhesion in Swiss albino mice (Table.1). The treatment induced marked enhancement of humoral and DTH response in the animals (Table.2). From the study it may be inferred that Cdpf promotes immunomodulation and thus rationalizing its traditional claim.

### Table 1: Effect of Cdpf on neutrophil adhesion in Swiss albino mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TLC(10^6/mm^3)</th>
<th>Neutrophil (%)</th>
<th>Neutrophil Index</th>
<th>Neutrophil adhesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogallol</td>
<td>5.95±0.37</td>
<td>28.10±2.48</td>
<td>25.44±2.63</td>
<td>249.25±21.56</td>
</tr>
<tr>
<td>PBS</td>
<td>11.45±0.34</td>
<td>29.16±2.48</td>
<td>26.50±1.87</td>
<td>333.73±24.38</td>
</tr>
<tr>
<td>Cdpf</td>
<td>10.86±0.65</td>
<td>29.16±2.48</td>
<td>27.83±3.18</td>
<td>316.60±30.21</td>
</tr>
</tbody>
</table>

All the values are mean±SD of mice in each group. One-way ANOVA followed by Turkey-Kramer comparisons test, p<0.05 VS Group1, F (2, 15) =9.4, TLC= Total Leukocyte Count; UB= Untreated blood; FTB= Fiber Treated Blood
**Table 2: Effect of Cdpf on HA titre and DTH response to antigenic challenge by sheep RBCs in mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HA Titre</th>
<th>DTH response (% increase in paw volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogallol</td>
<td>1:16</td>
<td>9.6±2.66</td>
</tr>
<tr>
<td>PBS</td>
<td>1:32</td>
<td>10.32±1.56</td>
</tr>
<tr>
<td>Cdpf</td>
<td>1:64</td>
<td>22.5±6±5.54</td>
</tr>
</tbody>
</table>

All the values are mean±SD of mice in each group. One-way ANOVA followed by Turkey-Kramer comparisons test. p<0.05 VS Group1, F (2, 15) =4.1) for HA titre and F (2, 15) =10.2) for DTH response

**REFERENCES**

13. Mitra SK, Gupta M and Sarma DNK. Immunomodulatory effect of Immunostimulant activity of Cdpf was known there was no documentary evidence. In conclusion, the results obtained in the present study have shown the immunomodulatory activity of Cdpf in vivo, further studies are warranted for the understanding the exact mechanisms responsible for immunomodulation.

**CONCLUSIONS**

*Cynodon dactylon* has shown significant immummodulatory effect in animals. Therefore clinical studies as a potential immunostimulant is further warranted. Its efficacy as antitumor agent has been demonstrated. It is assured as it has been consumed as drink from centuries. Therefore, we assume that clinical studies with *Cynodon dactylon* in immunomodulation will result in positive outcome.

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