Formulation and evaluation of herbal gel

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The present study has been undertaken with the aim to formulate and evaluate the polyherbal gel containing Cassia alata Linn., Cassia tora Linn. and Cynodon dactylon Pers. extract. The gel formulation was designed by using methanolic extract of leaves of C. alata and C. tora and aerial part of C. dactylon in varied concentrations (1, 2 and 4%). Topical anti-inflammatory activity of gel was also evaluated. The gel was prepared by using Carbopol 940 (1% w/v), C. alata, C. tora and C. dactylon extract, ethanol, propylene glycol 400, methyl paraban, propyl paraben, EDTA, tri-ethanolamine and required amount of distilled water. The prepared gels were evaluated for physical appearance, pH, spread ability, skin irritation to observe toxicity or side effects and also for anti inflammatory activity. It was inferred from the results that gel formulations were good in appearance and homogeneity. The values of spread ability indicated that these polyherbal gels were easily spreadable by small amount of shear. Viscosity of polyherbal gels were determined by using Brookfield viscometer and were ranging between 4500 to 4900 centipoise. The gels showed significant inhibition in carrageenan induced paw oedema and formalin induced paw oedema in Wistar rat models.

Keywords: Anti-inflammatory, Carbopol 940, Cassia alata, Cassia tora, Cynodon dactylon, Herbal gel, Topical.

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Introduction

Topical application of gels at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments1,2. Cassia alata Linn. and C. tora Linn. (Family: Caeasalpiniaceae) is phytochemically rich in steroids, alkaloids, tannins, triterpenes, flavanoids and antraquinone glycoside. Both C. tora and C. alata have been found to be used traditionally for their various therapeutic properties like, anticancer3 and oral anti-inflammatory4,5, antibacterial6, antioxidant7, skin disorder and wound-healing activities8. The plant Cynodon dactylon Pers. (Family: Poaceae) is phytochemically rich in steroids, tannins, flavonoids, carbohydrates and alkaloids and has been found to be used traditionally for various therapeutic properties like, antiviral9, antidiabetic10, antifungal11, antibacterial12, antioxidant13, antiulcer14 skin disorder and wound-healing activities15,16. The present study was undertaken to evaluate the topical anti-inflammatory potential of C. alata, C. tora and C. dactylon.

The present investigation involves the preparation of gel formulations of C. alata, C. tora and C. dactylon (methanolic extract in varied concentration) followed by the evaluation for physical appearance, pH, viscosity, spread ability and for anti-inflammatory activity.

Materials and Methods

Plant collection and authentication

The leaves of Cassia tora and C. alata were collected in August 2010 at Hingna, MIDC area of Nagpur. The aerial part of Cynodon dactylon was collected from medicinal plant garden of J. L. Chaturvedi college of Pharmacy, Nagpur. The collected plants were authenticated at Department of Botany, R.T. M. Nagpur University, Nagpur. Voucher specimens (9549, 9550 and 9551) are maintained in the Department.

Chemicals

Carbopol 934 (Merck Ltd), Diclofenac sodium (Zim lab India), Ethanol (Loba Chem India), Methyl Paraban (Loba Chem India), Propyl Paraben (Loba Chem India), Propylene glycol-400 (Loba Chem India), Tri-ethanolamine (Loba Chem India), Carragennan (Sigma Aldrich), Formalin (Loba Chem India) were purchased from the market.

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Preparation of plant extracts

The collected fresh leaves of *C. alata*, *C. tora* and aerial parts of *C. dactylon* were dried in hot air oven at 40°C to avoid degradation of phytoconstituents. After drying plant materials were coarsely powdered with Willey mill and kept in well closed container. About 185, 100 and 125 g powder of each specimen, respectively were defatted with Pet. ether (60-80°C) in Soxhlet apparatus. After defatting, it was further extracted with methanol. The collected extracts were concentrated on rotary evaporator and concentrated extracts were kept in vacuum dryer until used.

Animals

The Wister rats weighing between 150-200 g were procured from Animal house of J. L. Chaturvedi College of Pharmacy, R.T.M. Nagpur University, Nagpur and maintained under constant conditions (temperature 25± 2°C, humidity 40-60%, 12 h light/12 h dark cycle). During maintenance the animals received a diet of food pellet supplied from animal house and water *ad libitum*. These experiments were approved by the Institutional Animal Ethics Committee, R.T.M. Nagpur University, Nagpur (IAEC No. 648/02/6/CPCSEA).

Preparation of topical gels

The gel was prepared using the dried methanolic extract of *Cassia alata*, *C. tora* and *Cynodon dactylon*. The gel was prepared using Carbapol-940 (1%), propylene glycol 400, ethanol, methyl paraben, propyl paraben, EDTA, tri-ethanolamine and distilled water. Both of these solutions were added to this solution methyl paraben, propyl paraben and EDTA was added. Both of these solutions were mixed in a beaker and tri-ethanolamine was added to the mixture dropwise to obtain the gel consistency. The same procedure was used for preparation of Diclofenac sodium gel as standard. Composition of gel formulation is given in Table 1.

Evaluation of gel formulations

Evaluation parameters of gel formulations are given in Table 2 and they are:

- **pH** — The pH value of polyherbal gel formulation was determined by using a pH meter. The measurement was performed at 1, 30, 60, 90 days after preparation to detect any pH changes with time.
- **Appearance and Homogeneity** — All developed gels were tested for physical appearance and homogeneity by visual observation.
- **Viscosity** — The measurement of viscosity of the prepared polyherbal gel was done with Brookfield viscometer (Model RVTDV II). The reading was taken at 100 rpm using spindle no. 6.
- **Spread ability** — The spread ability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) after one minute. The standard weight applied on the upper plate was 125 g.
- **Skin irritation studies** — The Wistar rats of either sex weighing 150-200 g were used for this test. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The gels containing extracts were used on test animal. Gel base was applied on the back of animal taken as control. The animals were treated daily up to seven days and finally the treated skin was examined visually for erythema and edema.

Assessment of anti-inflammatory activity

Pedal inflammation in animal was produced according to the method described by Winter *et al* (1962)34. Rats were divided in 9 groups of six rats in each. Group I was applied with gel base and served as control. Group II standard (Diclofenac sodium Gel 0.5%) and served as reference. Group III-IX application of 1 g of 1, 2, 4% gel of *Cassia alata*, *C. tora* and *Cynodon dactylon* each, respectively.

The edema was induced by injecting 0.1 ml of carrageenan (1% w/v) in normal saline into the sub planter region of the left hind paw, after 1 hour of drug application. Paw thickness was measured with the help of Digital Vernier caliper at 0, 30, 60, 120, 180, 240 and 300 min after administration of

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Carbap-940 (%)</th>
<th>Extract (%)</th>
<th>Propylene glycol (%)</th>
<th>Ethanol (%)</th>
<th>Methyl paraben (%)</th>
<th>Propyl paraben (%)</th>
<th>EDTA (%)</th>
<th>Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A., C.T., C.D. Gel 1%</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>Up to 100</td>
</tr>
<tr>
<td>C.A., C.T., C.D. Gel 2%</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>Up to 100</td>
</tr>
<tr>
<td>C.A., C.T., C.D. Gel 4%</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>Up to 100</td>
</tr>
</tbody>
</table>

carrageenan. The dosage details are presented in Table 3 and 4.

**Formalin-induced rat paw edema**

The formalin-induced rat paw edema model was used for acute as well as chronic inflammation on the basis of formalin concentration. For chronic model 2% of formalin in saline was used. Formalin-induced edema is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandin are known to be involved. The dosage details are given in Table 5.

The % inhibition of edema was calculated by formula:

\[
\% \text{Inhibition} = 1 - \frac{(\alpha - \chi)(\beta - \gamma)}{100}
\]

where,

- \(\alpha\) = paw thickness of test animal after treatment
- \(\beta\) = paw thickness of control animal after treatment
- \(\chi\) = initial paw thickness of test animal
- \(\gamma\) = initial paw thickness of control animal.

### Results and Discussion

The dried powder of leaves and aereal part were defatted with petroleum ether (60-80°C) and extracted with methanol. The average % practical yield of methanolic extract of *C. alata, C. dactylon* and *C. tora* were found to be 18.41, 12.30 and 8.152%, respectively. Polyherbal gels of methanolic extract of *C. alata* (1%), *C. tora* (2%) and *C. dactylon* (4%) are prepared by using Carbapol-940. Composition of herbal gels is shown in Table 1. The physicochemical properties of the gel formulation were shown in Table 2. From the results it is concluded that all the gel formulations showed good appearance and homogeneity. The physical appearance of the gel formulations was green in nature. The pH of the gel formulations was in the range of 6.15±0.04 to 6.98±0.03, which lies in the normal pH range of the skin and with time no skin irritation was observed. There was no significant change in pH values with time (varied from 0.05 to 0.20). Viscosity of polyherbal gels were determined by using Brookfield viscometer and were ranging between 4700 to 4800 cp.

### Statistics

All values are expressed as mean ± SEM. *P* value calculated by comparing with control by ANOVA followed by Bonferroni post test. *P* values lower than 0.05 were considered significant.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Appearance</th>
<th>Homogeneity</th>
<th>Spreading Diameter after 1 min (mm)</th>
<th>Viscosity (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclo gel</td>
<td>6.15</td>
<td>White</td>
<td>Good</td>
<td>55</td>
<td>4500</td>
</tr>
<tr>
<td>C.A. 1%,</td>
<td>6.35</td>
<td>Light green</td>
<td>Good</td>
<td>48</td>
<td>4700</td>
</tr>
<tr>
<td>C.T. 1%</td>
<td>6.30</td>
<td>Light green</td>
<td>Good</td>
<td>42</td>
<td>4700</td>
</tr>
<tr>
<td>C.D. 1%</td>
<td>6.31</td>
<td>Light green</td>
<td>Good</td>
<td>44</td>
<td>4800</td>
</tr>
<tr>
<td>C.A. 2%</td>
<td>6.45</td>
<td>Light green</td>
<td>Good</td>
<td>42</td>
<td>4700</td>
</tr>
<tr>
<td>C.T. 2%</td>
<td>6.50</td>
<td>Light green</td>
<td>Good</td>
<td>40</td>
<td>4700</td>
</tr>
<tr>
<td>C.D. 2%</td>
<td>6.49</td>
<td>Light green</td>
<td>Good</td>
<td>42</td>
<td>4800</td>
</tr>
<tr>
<td>C.A. 4%</td>
<td>6.45</td>
<td>Light green</td>
<td>Good</td>
<td>40</td>
<td>4700</td>
</tr>
<tr>
<td>C.T. 4%</td>
<td>6.46</td>
<td>Light green</td>
<td>Good</td>
<td>39</td>
<td>4800</td>
</tr>
<tr>
<td>C.D. 4%</td>
<td>6.48</td>
<td>Light green</td>
<td>Good</td>
<td>41</td>
<td>4800</td>
</tr>
</tbody>
</table>

Table 3 — Anti-inflammatory activity of different gel formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Control (Gel base)</th>
<th>Initial/ 0 h</th>
<th>½ h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cassia alata Gel</strong></td>
<td></td>
<td>5.11±0.027</td>
<td>8.10±0.021</td>
<td>8.36±0.012</td>
<td>8.89±0.029</td>
<td>9.21±0.022</td>
<td>8.94±0.016</td>
<td>7.92±0.026</td>
</tr>
<tr>
<td>Control Standard</td>
<td></td>
<td>5.20±0.026</td>
<td>5.88±0.023***</td>
<td>6.14±0.023***</td>
<td>6.65±0.027**</td>
<td>6.97±0.021***</td>
<td>6.70±0.012***</td>
<td>5.80±0.026***</td>
</tr>
<tr>
<td><strong>Cynodon dactylon Gel</strong></td>
<td></td>
<td>5.11±0.020</td>
<td>6.18±0.026***</td>
<td>6.24±0.027***</td>
<td>6.96±0.029**</td>
<td>7.95±0.027***</td>
<td>7.11±0.024***</td>
<td>6.25±0.013***</td>
</tr>
<tr>
<td>1%</td>
<td></td>
<td>5.05±0.017</td>
<td>6.18±0.026***</td>
<td>6.21±0.033***</td>
<td>6.94±0.024**</td>
<td>7.84±0.027***</td>
<td>7.08±0.013***</td>
<td>6.04±0.026***</td>
</tr>
<tr>
<td>2%</td>
<td></td>
<td>5.05±0.024</td>
<td>6.17±0.026***</td>
<td>6.21±0.022***</td>
<td>6.90±0.021**</td>
<td>7.25±0.027***</td>
<td>7.02±0.022***</td>
<td>5.81±0.016***</td>
</tr>
<tr>
<td>4%</td>
<td></td>
<td>5.02±0.045</td>
<td>6.64±0.033***</td>
<td>6.89±0.055***</td>
<td>7.45±0.061**</td>
<td>7.77±0.055***</td>
<td>7.16±0.038***</td>
<td>6.27±0.044***</td>
</tr>
<tr>
<td><strong>Cassia tora Gel</strong></td>
<td></td>
<td>5.11±0.031</td>
<td>6.18±0.045***</td>
<td>6.51±0.045***</td>
<td>7.15±0.072**</td>
<td>7.69±0.059**</td>
<td>6.98±0.040**</td>
<td>6.23±0.044**</td>
</tr>
<tr>
<td>1%</td>
<td></td>
<td>5.05±0.031</td>
<td>6.16±0.051***</td>
<td>6.46±0.051***</td>
<td>6.91±0.053**</td>
<td>7.15±0.057***</td>
<td>7.00±0.039**</td>
<td>5.82±0.044***</td>
</tr>
<tr>
<td>2%</td>
<td></td>
<td>5.04±0.026</td>
<td>6.75±0.152***</td>
<td>7.02±0.025**</td>
<td>7.55±0.026**</td>
<td>7.85±0.026**</td>
<td>7.43±0.027**</td>
<td>6.58±0.030***</td>
</tr>
<tr>
<td>4%</td>
<td></td>
<td>5.05±0.026</td>
<td>6.23±0.044***</td>
<td>6.68±0.062**</td>
<td>7.15±0.053**</td>
<td>7.21±0.060**</td>
<td>6.63±0.026**</td>
<td>6.05±0.040***</td>
</tr>
</tbody>
</table>

n: Number of animals. (n= 6)

(i) 1 g of formulation was applied to the planter surface of the right hand paw by gently rubbing 50 times with index finger.

(ii) Values are mean ± S.E.M. *P< 0.05, **P< 0.01, ***P< 0.001. *P* value calculated by comparing with control by ANOVA followed by Bonferroni post test.
centipoise. The values of spread ability indicated that the gels were easily spreadable by small amount of shear. Spreading diameter after 1 min (mm) of prepared gel was between 38-55 mm which indicates good spread ability of polyherbal gels. The results of skin irritancy studies indicated that the prepared gels were free from dermatological reaction. The gels were evaluated for anti-inflammatory activity by using carrageenan-induced rat paw edema and formalin-induced rat paw edema topicaly. Statistical analysis showed that the edema inhibition by formulation containing extracts were significantly differing from control group at all the concentration tested. The results showed that the anti-inflammatory effect of the formulation containing 4% of C. alata gel was better than the effect of other gel formulation (Tables 3 & 4). The highest inhibition was found at 300 min. post carrageenan injection, which is supposed to be due to inhibition of late phase mediators, arachidonic acid product and prostaglandins, of acute inflammation induced by carrageenan. Formalin-induced rat paw edema model was used for acute as well as chronic inflammation on the basis of formalin concentration. For chronic model, 2% of formalin in saline is used. Statistical analysis showed that the edema inhibition by formulation containing extracts were significantly differing from control group at all the concentration tested. As compared to acute inflammation, in chronic model, it showed no significant result as compared to standard. The results showed that the anti-inflammatory effect of the formulation containing 4% of C. alata gel was better than the effect of other gel formulation (Table 5).

**Conclusion**

It is inferred from results that the gel formulations are good in appearance, homogeneity and easily spreadable and showed significant inhibition in carrageenan induced and formalin induced paw oedema in Wistar rat models. The results also showed that the anti-inflammatory effect of the formulation containing 4% of C. alata gel was better than the effect of other gel formulation.

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**References**

MISAL et al: FORMULATION AND EVALUATION OF HERBAL GEL