COMPARATIVE STUDIES ON ANTI-INFLAMMATORY ACTIVITY OF HYDROGELS CONTAINING HERBAL EXTRACTS

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ABSTRACT
The main objective of this study is to evaluate and compare anti-inflammatory activity of hydrogels containing herbal hydro-alcoholic extracts of Pterocarpus marsupium, Pterocarpus santalinus and Glycyrrhiza glabra. Hydroalcoholic solvent ethanol: water in 70:30 proportions was used for the extraction by continuous hot extraction using Soxhlet apparatus. The herbal hydrogels containing 10% extract as API were formulated using chitosan as gel base, by chemical crosslinking of chitosan with glutaraldehyde. Anti-inflammatory activity of herbal hydrogels was evaluated by carrageenan induced rat hind paw edema method. Animals were divided into six group comprising six animals in each group of either sex, for comparing their activity against standard (Enacgel) and control groups. The hydrogel containing hydro-alcoholic extracts of Pterocarpus marsupium showed significant anti-inflammatory activity (43.70%) when compared with the standard (17.03%) at the end of 8 hrs and is more significant as that of marketed formulation.

Keywords: Anti-inflammatory activity, hydrogel, Pterocarpus marsupium, hind paw method.

INTRODUCTION
Now-a-day’s interest in natural products as potent therapeutic agent has increased tremendously, the modern clinicians are now inclined towards the use of herbal medicine1-3. WHO estimate that 65-80% of the world population uses traditional medicine as their primary form of healthcare and about 85% of the traditional medicine involve the use of herbal preparation fully aware of the importance of the herbal medicine as a valuable answer readily available resources for primary health care and has endorsed there safe and effective use4.

Inflammation induced pain have affected mankind for ages. Their crippling and incapacitating effect on the affected patient present many emotional, social and economical problems. Despite lot of research and effective cure has still eluded us. All that can be offered are the anti-inflammatory and pain killer like NSAID. Ayurvedic literature is full of herb for solving this type of pain. From old times people tried to heal inflammation. They used crude drug extracts (mostly of plant origin), animal fat and honey to heal inflammation, Vijaysar (Pterocarpus marsupium), Red Sandalysar (Pterocarpus santalinus), P. marsupium has also shown strong potential for its antiinflammatory activity. An extract of P. marsupium and P. santalinus containing pterostilbene has been evaluated for its PGE2- inhibitory activity in LPS-stimulated PBMC. In addition, the COX-1/2 selective inhibitory activity of P. marsupium extract.
was investigated. Glycyrrhiza glabra belonging to family Leguminosae contains Glycyrrhizin which on hydrolysis gives glycyrrhetinic acid which shows anti-inflammatory activity.

A gel is a soft, solid or solid-like material consisting of two or more components, one of which is a liquid, present in substantial quantity; gels may appear more solid like than liquid like. Hydrogels are also made up of hydrophilic polymer strands but they are crosslinked and that enable them to swell while retaining their three dimensional structure. Hydrogels are gels system in which water is immobilized by insoluble polymer. One reason for the interest in hydrogels as component of drug delivery system is their relatively good compatibility with biological tissue. Instead of conventional creams, the hydrogels have been formulated for better patient compliance. These hydrogels have moisturizing properties therefore scaling and dryness is not expected with this drug delivery system also it has shown better absorption than conventional cream formulations.

Chitosan has been utilized for many medical and pharmaceutical preparations. Properties such as being biocompatible, non-toxic and soluble in weak acids make it an excellent wound dressing. It is positively charged, is strong tissue adhesive and forms gel easily. It has proven to enhance function of leukocytes, macrophages and fibroblasts to enhance granulation and rebuilding tissue.

Chitosan hydrogels have been widely studied as topical formulations. Among others, Alsarra (2009) evaluated chitosan hydrogels in topical formulations. Different molecular weight and different degree of deacetylation ranges were compared. Although high molecular weight (HMW) chitosans will be more viscous than low molecular weight (LMW) one and the medium molecular weight (MMW) at the same concentration.

MATERIALS AND METHODS

The heartwood of Pterocarpus marsupium, Pterocarpus santalinus and stolons and roots of Glycyrrhiza glabra were purchased from Yucca Enterprises, Mumbai, and was authenticated from botanist. Chitosan was received as a gift sample from V. Kumar and Son’s. All other chemicals were of analytical grade and were supplied by different chemical suppliers.

All the herbal crude drugs were powdered and were extracted separately by continuous hot solvent extraction method using Soxhlet apparatus, the extract was collected and concentrated and dried in evaporating dish using heating mantle and powdered.

Preparation of Hydrogels

Four different formulations were prepared using chitosan as gel base. 2.85 gm of chitosan was dissolved in 50ml of lactic acid (2%) and was allowed to swell. 10% glycerin was added as humectant and as plasticizer, 10gm of extract was dissolved in 20ml of ethanol which also acts as penetration enhancer and was added in previous mixture and was stirred using mechanical stirrer for 5min, 0.3ml of glutaraldehyde (0.5%) solution was added and was stirred for half hour to allow cross linking of polymer, the pH of the formulation was maintained around 6 using triethanolamine, and the gels were allow to stand for 48 hrs to settle.

Anti-inflammatory Activity:

Albino rats of Wistar strain of either sex between 130-240 gm breed were selected for the studies. The animals were fastening overnight but allow water at libidum. The permission to carry out animal studies was obtained from CPCSEA and was approved
from Institutional Animal Ethics Committee of R. C P. Kasegaon [RCP/IAEC/2011-12/P-003]. The animals were divided into six groups comprising six animals of either sex in each group as,

<table>
<thead>
<tr>
<th>Group I (Control group)</th>
<th>For Control (co)</th>
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<tbody>
<tr>
<td>Group II (Standard group)</td>
<td>Marked formulation Enacgel.</td>
</tr>
<tr>
<td>Group III</td>
<td>Hydrogel base (No extract)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Hydrogel containing extract of G. glabra</td>
</tr>
<tr>
<td>Group V</td>
<td>Hydrogel containing extract of P. Marsupium</td>
</tr>
<tr>
<td>Group VI</td>
<td>Hydrogel containing extract of P. Santalinus</td>
</tr>
<tr>
<td>Group VII</td>
<td>Hydrogel containing mixture of these three extracts</td>
</tr>
</tbody>
</table>

The anti-inflammatory activity of formulated hydrogel will be evaluated by the carrageenan-induced rat hind paw edema method of Winter et al. 1 gm of the respective formulation (Hydrogels) was applied to the rat's paw 1 hour before carrageenan injection 0.1ml solution (1%) subcutaneously. The paw volume was noted at interval of one hour and accordingly activity of each formulation was evaluated in terms of % inhibition of edema using following formula.

\[
\text{% inhibition of edema} = \left(1 - \frac{V_t}{V_c}\right) \times 100
\]

Where,

\[
V_t = \text{Volume of edema in test} \\
V_c = \text{Volume of edema in control}
\]

RESULT

The formulated hydrogels of different extracts were evaluated using carrageenan induced rat hind paw edema method and anti-inflammatory activity in terms of percent inhibition of edema was calculated by comparing them with control group and is shown in table no: 1

DISCUSSION

Hydrogels prepared from an aqueous extract of these plants were quite stable. The stability might be due to antimicrobial properties of chitosan. Due to the penetration enhancer property of chitosan and ethanol anti-inflammatory effect were observed within few hours after carrageenan injection. The present study reveals that the activity of hydrogel containing \( P. marsupium \) is significant as compared to the marketed formulation. Hydrogel containing chitosan as gel base enhances activity further.

Hydrogel F 2 containing \( G. glabra \) extract shows 50% inhibition of edema after 1hr, while F4 and F5 showed 60% inhibition of edema. F 3 behaves more significant effect than F2, F4 and F5 and showed about 80% inhibition of edema as equivalent as formulation containing standard. F1 having only chitosan (gel base) produces near about 25 % inhibition of edema. F5 indicates 60% inhibition of edema, which is less significant as compare to standard, while F2 containing \( P. marsupium \) showed more significant effect as compare to others. After 2hrs F2, F4 and F5 exhibit about 60% inhibition of edema, while F3 and F6 have better anti-inflammatory effect. The result reveals that all the herbal hydrogels potentiates good activity in first 2 hrs after application of hydrogels and then after 3hrs the % inhibition of edema decreases till 7 to 8 hrs. Thus chitosan can potentiates the anti-inflammatory activity of all herbal extracts with sustain release of medicament. After 8hr F3 containing \( P. marsupium \) pointed a better anti-inflammatory effect than other formulations. It emphasize that \( P. marsupium \) with chitosan is having synergistic effect on % inhibition of edema as compared to standard gel or any synthetic one.

Form the table no.1, fig no.1 and figno.2 it is postulated that the herbal hydrogels gives better activity in first 3 hrs due to potent target molecules of extract and penetration enhancers as ethyl alcohol and chitosan, after 2hrs to 5hrs the activity decrease due to unavailability of ethyl alcohol as it is volatile. The activity again increases up to 8hrs because there may be chances of total combined effect of all constituents in extract and total release of medicament as well as chitosan at the site of action.

The present research work peak points minimum % inhibition of edema of hydrogel containing only chitosan as gel base. Chitosan with \( P. marsupium \) have a wonderful anti-
inflammatory effect in animal study as compare to *P. Santalinus, G. glabra*, mixture of extract and standard diclofenac gel. Chitosan itself acts as penetration enhancer, antibacterial, analgesic and anti-inflammatory therefore it is use in various external applications and is the best alternative to other synthetic polymers for to use as gelling agents. As chitosan and herbal extracts are natural its widespread use in the form of hydrogel will be new vistas for herbal industry to produce a well acceptable, palatable and elegant preparation to meet consumer's needs. It is necessary to investigate molecules of all these crude drugs which are responsible for anti-inflammatory activity. It is possible for researchers, to formulate herbal extracts in hydrogel and such other semisolid dosage form as an external application and to evaluate them.

**Table 1: Anti-inflammatory effect in terms of percent inhibition of edema**

<table>
<thead>
<tr>
<th>Time in hrs</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>15.43%</td>
<td>49.66%</td>
<td>79.86%</td>
<td>59.73%</td>
<td>59.73%</td>
<td>80.53%</td>
</tr>
<tr>
<td>2</td>
<td>24.99%</td>
<td>62.8%</td>
<td>80.48%</td>
<td>64.02%</td>
<td>68.9%</td>
<td>88.01%</td>
</tr>
<tr>
<td>3</td>
<td>9.36%</td>
<td>23.26%</td>
<td>45.01%</td>
<td>37.76%</td>
<td>46.22%</td>
<td>47.42%</td>
</tr>
<tr>
<td>4</td>
<td>5.76%</td>
<td>14.74%</td>
<td>38.46%</td>
<td>25%</td>
<td>33.33%</td>
<td>35.25%</td>
</tr>
<tr>
<td>5</td>
<td>3.82%</td>
<td>13.96%</td>
<td>23.52%</td>
<td>13.23%</td>
<td>13.96%</td>
<td>22.05%</td>
</tr>
<tr>
<td>6</td>
<td>3.21%</td>
<td>18.97%</td>
<td>22.63%</td>
<td>21.16%</td>
<td>13.86%</td>
<td>19.7%</td>
</tr>
<tr>
<td>7</td>
<td>2.33%</td>
<td>21.89%</td>
<td>32.11%</td>
<td>26.27%</td>
<td>17.51%</td>
<td>18.97%</td>
</tr>
<tr>
<td>8</td>
<td>2.22%</td>
<td>24.44%</td>
<td>43.7%</td>
<td>33.33%</td>
<td>26.66%</td>
<td>17.03%</td>
</tr>
</tbody>
</table>

**Fig. 1: Average % edema inhibition by different hydrogel formulations**
CONCLUSION
Animal study showed that formulation F3 containing extract of *P. marsupium* shows higher anti-inflammatory activity as compared to the F6 (marketed formulation enacgel) as well as F2, F4, and F5 formulation. Formulation containing extract of *P. marsupium* could be used as an effective formulation for to heal the inflammation as compared to formulation containing extract of *G. glabra, P. santalinus* and the mixture of extract and the marketed one.

ACKNOWLEDGEMENTS
The authors are thankful to V. Kumar and Son’s for providing chitosan as gift sample and also to, The Principal Rajaramabapu College of Pharmacy for providing facilities to research work.

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Fig. 2: Average % edema inhibition by different hydrogel formulations (Graph)