DEVELOPMENT AND EVALUATION OF POLYHERBAL OINTMENT FOR HAIR GROWTH PROMOTING ACTIVITY

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ABSTRACT

Alopecia is one of the dermatological disorders a global problem, affecting both genders of all races. The present study is an effort to formulate and investigate hair growth promoting activity of polyherbal ointment. Polyherbal ointment formulation was prepared by fusion method. The ethanolic extracts of *Piper betle* (Leaves), *Chrysanthemum indicum* (Flowers), *Ficus benghalensis* (Roots), *Ziziphus mauritiana* (Leaves) and *Thymus vulgaris* (Leaves) (5% w/w) was incorporated in hydrophilic USP base. Primary skin irritation test was performed on rats and all the prepared formulations are safe from producing erythema or edema. The ointments were applied topically on shaved skin of rats for 30 days. Hair length, hair density and total protein estimations were carried out. Obtained results revealed that polyherbal ointment shown significant hair growth as compare to single extracts ointments. Flavonoids, tannins present in the polyherbal ointment may be responsible for hair growth promoting activity. Lastly we conclude that the formulated ointment is promising and even better results are expected with variation in the proportion of these drugs. Further studies have to be emphasized on isolating the bioactive molecules in the polyherbal ointment responsible for hair growth promotion.
KEYWORDS: Piper betle, Chrysanthemum indicum, Ficus benghalensis, Ziziphus mauritiana and Thymus vulgaris.

1. INTRODUCTION

Hairs are protective appendages on the body derived from ectoderm of skin and considered as accessory structure of the integument along with sebaceous glands; sweat glands and nails.[1] During embryological development hairs originate from the epidermis hence known as epidermal derivatives. 85% of all the hairs are in the Anagen phase (Growing phase) at any one time varying from two to six years.[2] After the Anagen phase the hairs enters into a Catagen phase and in this phase the hair follicle shrinks to about 1/6 of the normal length.[3] which lasts about one or two weeks, The Telogen phase (Resting phase) follows the catagen phase and usually lasts about 5-6 weeks. During this time the hair does not grow but remains attached to the follicle while the dermal papilla stays in a resting phase below.

Alopecia is one of the dermatological disorders a global problem, affecting both genders of all races. The major problems associated with hair are Fading, dandruff and Shedding. Excess androgens production such as dihydrotestosterone, iron deficiency anaemia, stress, protein energy malnutrition, nervous disorders, aging, hormonal imbalance and exposure to radiations are the confounding factors contributing to hair loss.[4] Minoxidil a potent vasodilator is used for treatment of alopecia. As synthetic drugs are linked with side effects minoxidil usage is limited besides its pharmacological benefits. India is a depot of medicinal plants and in traditional system of medicine, numerous herbal formulations are reported for hair growth promotion but lack of sound scientific backing and information limits their use.[5] Hence the present study is an effort to formulate and evaluate hair growth promoting activity of polyherbal ointment.

2. MATERIAL AND METHODS

2.1. Collection, identification and authentication of plants

Plant material of Piper betle (Leaves), Chrysanthemum indicum (Flowers), Ficus benghalensis (Roots), Ziziphus mauritiana (Leaves) and Thymus vulgaris (Leaves) were collected in the month of January-February from the region of Nalgonda and were identified and authenticated by pharmacognosist- Karnati. Sushma, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda and a voucher specimen (No:SRTIPS/COG/2345) was deposited in department of Pharmacognosy. The collected plant material was shade dried thereafter reduced to powder form.
2.2. Preparation of extract

The powdered material (1000g) was extracted in a Soxhlet apparatus with Ethanol (60 – 80°C) for 18 hours. The extract was filtered, dried and subjected to preliminary phytochemical screening.

2.3. Preliminary Phytochemical Screening

Preliminary qualitative phytochemical analysis for all the obtained ethanolic extracts was carried out by employing standard conventional protocols.[6-8]

2.4. Formulation

Formulation was prepared by fusion method. The ethanolic extracts of all the above mentioned herbs are incorporated into ointment base maintaining concentration as 5% (w/w). All the ointments are prepared by following the procedure mentioned in United States Pharmacopeia (USP). Hydrophilic ointment USP contains methyl paraben 0.25, propyl paraben 0.15, propylene glycol 120.0, stearyl alcohol 250.0; white petrolatum 250.0, Sodium Lauryl sulphate (SLS) 10.00, purified water 370.0 and different herbs extracts to make about 1000g. The stearyl alcohol and white petrolatum are melted together at above 75°C, the other agent with herb extract dissolved in the purified water, were added with stirring until the mixture gets congealed. SLS act as emulsifying agent, while the stearyl alcohol and white petrolatum constitute the oleaginous phase of emulsion and other ingredients of aqueous phase. Methyl and propyl paraben act as antimicrobial preservatives.[9]

2.5. Skin irritation test

Preliminary skin irritation test was performed on albino rats; the skin from the back of six rats was shaved using marketed hair removal cream (Veet). On cleaned test areas, the prepared formulations are applied and observations were made visually for the appearance of irritation or erythema for a period of 72 hr after the application of test preparations.[10]

2.6. Evaluation for hair growth activity in vivo

Female wistar albino rats, 200-250gm, are utilized for hair growth studies. They were fed with standard diet and allowed free access to drinking water. The experimental protocol (No: SRTIPS/FM/1468/PO/a/11/CPCSEA/117/2017) was duly approved by institutional animal ethics committee (IAEC).
2.6.1. Animal Grouping
Fourty eight albino rats were divided into eight groups of six animals in each group. Hairs from a 3 cm² area at the dorsal portion of all the rats were shaved using marketed hair removal cream to completely remove hair. Group 1 serves as a control applied with simple ointment. Group 2 serves as standard applied with 1ml of 2% minoxidil. Groups 3–7 will be topically applied with PB, CI, FB, ZM, TV ointments respectively. Group 8 will be applied with poly herbal ointment (PB+CI+FB+ZM+TV) over the shaved area. All the ointments and standard drug were applied once in a day. The treatment was continued for 30 days and hair growth pattern was observed and tabulated.

2.6.2. Hair length determination
Hair was plucked randomly using forceps from the shaved area of rats on 30th days of treatment. Hair length was measured and the results were recorded as mean length ± SEM of 25 hairs.\textsuperscript{[11]}

2.6.3. Hair density
A hole of 1 cm² was made on cardboard. Then the cardboard set on the desired depilated area on the back of rat after 30 days of depilation. The hair was trimmed of desired depilated area and the hair was cut with the scissor. The hairs are counted manually.\textsuperscript{[12]}

2.6.4. Total protein estimation
Total serum protein in blood will be estimated by Modified Biuret method. Biuret is a compound formed by heating urea to 180°C. When biuret treated with diluted copper sulfate in medium, a purple colure is obtained.\textsuperscript{[13]}

3. RESULTS
3.1. Preliminary Phytochemical Screening
Preliminary phytochemical analysis of ethanolic extract of \textit{Ficus benghalensis} and \textit{Chrysanthemum indicum} revealed the presence of flavonoids, saponins, steroids and tannins. \textit{Thymus vulgaris} showed the presence of flavonoids. \textit{Piper betle} showed the presence of tannins, anthraquinones, flavonoids, cardiac glycosides and alkaloids. \textit{Ziziphus mauritiana} revealed the presence of flavonoids, saponins and glycosides.
3.2. Primary skin irritation test

Primary skin irritation test was conducted to assess the irritation produced by the prepared formulations on intact skin of rats. All the prepared formulations are safe from producing erythema or edema.

3.3. Hair Length

Polyherbal formulation treated group (group-8) had shown significantly (P< 0.01) increased hair length compared to control. The results are depicted in Table 1& “Fig. 1”.

3.4. Hair Density

Polyherbal formulation treated group (group-8) had shown significantly (P< 0.01) increased hair density compared to control. The results are represented in Table 2 & “Fig. 2”.

3.5. Total Protein estimation

Polyherbal formulation treated group (group-8) had shown significantly (P< 0.01) increased protein level compared to control. The results are represented in Table 3 & “Fig. 3”.

Table 1: Effect of different ointments formulations on Hair length.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Treatment</th>
<th>Formulation</th>
<th>Hair length in mm (Mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group-1</td>
<td>Negative control: Simple ointment</td>
<td>Ointment base</td>
<td>1.36±0.25</td>
</tr>
<tr>
<td>2.</td>
<td>Group-2</td>
<td>Positive control- Minoxidil</td>
<td>Ointment (5%w/w)</td>
<td>3.05±0.29</td>
</tr>
<tr>
<td>3.</td>
<td>Group-3</td>
<td>Ethanolic Extract of <em>Piper betle</em> (PB)</td>
<td>Ointment (5%w/w)</td>
<td>1.98±0.22</td>
</tr>
<tr>
<td>4.</td>
<td>Group-4</td>
<td>Ethanolic Extract of <em>Chrysanthemum indicum</em> (CI)</td>
<td>Ointment (5%w/w)</td>
<td>2.53±0.35</td>
</tr>
<tr>
<td>5.</td>
<td>Group-5</td>
<td>Ethanolic Extract of <em>Ficus benghalensis</em> (FB)</td>
<td>Ointment (5%w/w)</td>
<td>2.11±0.28</td>
</tr>
<tr>
<td>6.</td>
<td>Group-6</td>
<td>Ethanolic Extract of <em>Ziziphus mauritiana</em> (ZM)</td>
<td>Ointment (5%w/w)</td>
<td>2.45±0.24</td>
</tr>
<tr>
<td>7.</td>
<td>Group-7</td>
<td>Ethanolic Extract of <em>Thymus vulgaris</em> (TV)</td>
<td>Ointment (5%w/w)</td>
<td>2.66±0.26</td>
</tr>
<tr>
<td>8.</td>
<td>Group-8</td>
<td>Polyherbal ethanolic extract of (PB+CI+FB+ZM+TV)</td>
<td>Ointment (5%w/w)</td>
<td>3.73±0.32</td>
</tr>
</tbody>
</table>
Table 2: Effect of different ointments formulations on Hair density.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Treatment</th>
<th>Formulation</th>
<th>Hair Density in mm (Mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-1</td>
<td>Negative control: Simple ointment</td>
<td>Ointment base</td>
<td>1163±70.67</td>
</tr>
<tr>
<td>2</td>
<td>Group-2</td>
<td>Positive control- Minoxidil</td>
<td>Ointment (5%w/w)</td>
<td>2898±130.2</td>
</tr>
<tr>
<td>3</td>
<td>Group-3</td>
<td>Ethanolic Extract of <em>Piper betle</em> (PB)</td>
<td>Ointment (5%w/w)</td>
<td>1527±31.57</td>
</tr>
<tr>
<td>4</td>
<td>Group-4</td>
<td>Ethanolic Extract of <em>Chrysanthemum indicum</em> (CI)</td>
<td>Ointment (5%w/w)</td>
<td>1919±41.40</td>
</tr>
<tr>
<td>5</td>
<td>Group-5</td>
<td>Ethanolic Extract of <em>Ficus benghalensis</em> (FB)</td>
<td>Ointment (5%w/w)</td>
<td>1664±36.49</td>
</tr>
<tr>
<td>6</td>
<td>Group-6</td>
<td>Ethanolic Extract of <em>Ziziphus mauritiana</em> (ZM)</td>
<td>Ointment (5%w/w)</td>
<td>1811±34.89</td>
</tr>
<tr>
<td>7</td>
<td>Group-7</td>
<td>Ethanolic Extract of <em>Thymus vulgaris</em> (TV)</td>
<td>Ointment (5%w/w)</td>
<td>2004±48.98</td>
</tr>
<tr>
<td>8</td>
<td>Group-8</td>
<td>Polyherbal ethanolic extract of (PB+CI+FB+ZM+TV)</td>
<td>Ointment (5%w/w)</td>
<td>3222±144.0</td>
</tr>
</tbody>
</table>

Table 3: Effect of different ointments formulations on Total serum protein.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Treatments</th>
<th>Formulation</th>
<th>Total serum protein g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-1</td>
<td>Negative control: Simple ointment</td>
<td>Ointment base</td>
<td>3.86±0.80</td>
</tr>
<tr>
<td>2</td>
<td>Group-2</td>
<td>Positive control- Minoxidil</td>
<td>Ointment (5%w/w)</td>
<td>6.73±0.50</td>
</tr>
<tr>
<td>3</td>
<td>Group-3</td>
<td>Ethanolic Extract of <em>Piper betle</em> (PB)</td>
<td>Ointment (5%w/w)</td>
<td>4.86±0.27</td>
</tr>
<tr>
<td>4</td>
<td>Group-4</td>
<td>Ethanolic Extract of <em>Chrysanthemum indicum</em> (CI)</td>
<td>Ointment (5%w/w)</td>
<td>5.71±0.67</td>
</tr>
<tr>
<td>5</td>
<td>Group-5</td>
<td>Ethanolic Extract of <em>Ficus benghalensis</em> (FB)</td>
<td>Ointment (5%w/w)</td>
<td>5.02±0.50</td>
</tr>
<tr>
<td>6</td>
<td>Group-6</td>
<td>Ethanolic Extract of <em>Ziziphus mauritiana</em> (ZM)</td>
<td>Ointment (5%w/w)</td>
<td>5.08±0.59</td>
</tr>
<tr>
<td>7</td>
<td>Group-7</td>
<td>Ethanolic Extract of <em>Thymus vulgaris</em> (TV)</td>
<td>Ointment (5%w/w)</td>
<td>5.87±0.59</td>
</tr>
<tr>
<td>8</td>
<td>Group-8</td>
<td>Polyherbal ethanolic extract of (PB+CI+FB+ZM+TV)</td>
<td>Ointment (5%w/w)</td>
<td>7.27±0.49</td>
</tr>
</tbody>
</table>
Effect of different ointment formulations on hair length

**Figure-1.**

Effect of different ointment formulations on hair density

**Figure-2.**

Effect of different ointment formulations on Total serum protein

**Figure-3.**
4. DISCUSSION

Our experimental data suggests that poly herbal ointment formulation facilitated hair growth promoting activity in wistar rats. The ethanolic extract of Polyherbal formulation shown significant hair growth as compare to single drug extract of *Piper betle*, *Chrysanthemum indicum*, *Ficus bengalensis*, *Ziziphus mauritiana* and *Thymus Vulgaris*. Preliminary phytochemical analysis was carried out for selected plants which showed the presence of phytoconstituents like flavonoids, tannins, may be responsible for hair growth activity. It could be considered that the Flavonoids present in the polyherbal ointment may be responsible for hair growth promoting activity. It has been proposed that flavonoids strengthen the capillaries of the smaller blood vessels present in hair follicles, improves blood circulation, promotes angiogenesis, nourishes the hair follicles there by promoting hair growth.\(^{[14-15]}\) It is evident from the past studies that flavonoids stimulates telogen to enter into anagen phase, a process involved in hair growth and also resulted in increased expressions of growth factors which has stimulatory effects on hair growth such as insulin – like growth factor-1, vascular endothelial growth factors, keratinocyte growth factors and hepatocyte growth factors.\(^{[16-18]}\) Poly herbal formulation on topical application improved hair growth and was superior to standard (2% minoxidil solution). Minoxidil on extended use is associated with adverse effects. This paved way to search the natural products from plant origin possessing potential hair growth promoting activity.

5. CONCLUSION

In the present investigation, hair growth was precisely improved on topical application of poly herbal ointment and this ointment will be safe, effective and economical for managing hair fall problem and hair growth promotion. Lastly we conclude that the formulated polyherbal ointment is promising and even better results are expected with variations in the proportion of these drugs. Further studies have to be emphasized on isolating the bioactive molecule responsible for hair growth promotion.

6. ACKNOWLEDGEMENTS

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7. REFERENCES


