EVALUATION OF SAFETY AND HAIR GROWTH PROMOTING ACTIVITY OF TRICHUP CAPSULE

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ABSTRACT

Large number of people around the world is suffering from hair loss (alopecia) or hair thinning. Though hair loss is not a life threatening health issue it can make life distressful and can thereby affect physical and mental health of human being. To find solution of such problems, an attempt has been made to evaluate the hair growth promotion activity of a herbo-mineral Ayurvedic formulation, Trichup Capsule. Safety of drug was also evaluated by conducting acute toxicity study as per OECD guideline. For evaluation of hair growth promoting activity, selected animals were divided in to three groups where each group was consisting of six animals. TED and TED × 2 group was treated with Trichup Capsule at 171 mg/kg/day and 342 mg/kg/day, p.o., for 30 days respectively. Visual parameters, hair length, hair diameter and serum total protein were determined. At the end of the study, animals did not manifest any signs of toxicity or mortality. Treatment of Trichup Capsule showed significant effect on all visual parameters. Length and diameter of hairs were significantly increased in TED and TED × 2 groups. Serum total protein was also observed to be elevated in TED and TED × 2 groups in comparison to normal control. Present study data revealed that Trichup Capsule is safe and having significant hair growth promoting activity.

Keywords: Hair growth promoting activity, Herbo-mineral Ayurvedic formulation, Trichup Capsule, Acute toxicity study

INTRODUCTION

Hair is one of the vital parts of the body derived from ectoderm of the skin and is protective appendages on the body. They are also known as epidermal derivatives as they originate from the epidermis during embryological development. Hair has an important contribution to the overall appeal of the human body1-3. Large number of people around the world is suffering from hair loss (alopecia) or hair thinning. Though hair loss is not a life threatening health issue it can make life distressful and can thereby affect physical and mental health of human being. In present time, Minoxidil is one of the most commonly prescribed conventional medicines which is a potent vasodilator and scientifically proven for the treatment of alopecia. However it has also been reported having various side effects like itching, skin rashes and irritation, inflammation on scalp etc. Therefore, it is important to develop safe and effective drugs that prevent hair loss as well as enhance hair growth. In this respect, alternative medicine has attracted much sufficient interest, although it has not yet been included into mainstream of medical care, due to the limited scientific evidences.

Trichup Capsule is a proprietary Ayurvedic formulation which contains extract of Eclipta alba (Bhringraj) whole plant5, Centella asiatica (Mandukparni) whole plant8,9, Glycyrrhiza glabra (Yashthimadhu) root10,11, Hibiscus rosa-sinensis (Japa) flower12,13, Rasayana Churna (Classical Ayurvedic formulation)14,15 and powder of some more classical Ayurvedic formulations such as Saptamrit Loh1, Narsinh Churna5, Gandhak Rasayana1 and Muktasukti Bhasma16. This product is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. Majority of the ingredients of Trichup Capsule are well reported in Ayurvedic texts and scientific research publications for hair growth promoting, hair vitalizing and anti-oxidant properties. However, no such evidence was available which proves safety and efficacy of their combination. In the present study, an attempt was made to evaluate the acute toxicity study as well as hair growth promoting activity of Trichup Capsule in wistar rats.

MATERIALS AND METHODS

Experimental animals

The experiment protocol described in present study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: KB/11/240) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.: 238/CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult wistar rats weighing 180-230 g were used to study acute toxicity and hair growth promoting activity. Rats were housed in polypropylene cages, maintained under standardized condition (12-hour light/dark cycle, room temperature 22 ± 2°C and humidity 55 ± 5 %) and provided free access to ‘Sabardan’ pelleted diet and purified drinking water ad libitium. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

Drugs and chemicals

Sample of Trichup Capsule was provided by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. The test formulation (Trichup Capsule) was administered orally in a form of suspension by mixing with vehicle 1 % Sodium Carboxy Methyl Cellulose (SCMC). Dose of the test drug was fixed by extrapolating the human dose to laboratory animals, based on body surface area ratio as per the table of Paget and Barnes17.
Acute toxicity study
Healthy Wistar albino rats either sex (180-210 g) were divided into 2 groups of 6 animals. The test formulation was suspended in distilled water and administered by gavage (orally) at single dose of 2000 mg/kg to 1st group and single dose of 5000 mg/kg to 2nd group. The general behavior and mortality of the rats was continuously monitored after dosing at every 1 h period during first 24 h (with special attention given during the first 4 h) and then daily for 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was carried out as per OECD Guideline 423.

Hair growth promoting activity
The selected animals were divided into three groups where each group consisted of six animals.
Group-I (NC): Served as normal control and received vehicle Group-II (TED): Served as test drug (Trichup Capsule) treated group [Therapeutic Effective Dose (TED) – 171 mg/kg/day, p.o., for 30 days]
Group-III (TED × 2): Served as test drug (Trichup Capsule) treated group [Double of Therapeutic Effective Dose (TED × 2) – 342 mg/kg/day, p.o., for 30 days]

Hairs from the 3 sq cm area of dorsal side of the rats were removed using scissor and a commercially available hair remover cream for complete removal of hair. The shaved area was cleaned with surgical spirit. Group II and III were treated with Trichup Capsule at 171 mg/kg/day and 342 mg/kg/day, p.o., for 30 days respectively.

Visual parameters
Qualitative hair growth analysis was undertaken by visual observation of two parameters. Hair growth initiation time i.e. time taken to initiate hair growth on denuded skin region and hair growth completion time i.e. time taken to complete cover the denuded skin region with new hair.

Determination of hair length and diameter
Hairs were plucked randomly from the shaved area of animals on 10th, 20th and 30th day after beginning the treatment. The length and diameter of 18 hairs were measured and average was determined. The results are expressed as the Mean ± S.E.M of 18 hairs. The length of hair was measured with the help of scale and the diameter was measured by double-scale stage micrometer.

Serum total protein estimation
At the end of the treatment, 8-10 mL blood samples were collected from retro-orbital plexuses of rats in clean dry centrifuge tubes under light ether anesthesia. Samples were allowed to clot for 30 minutes at room temperature. Sera from the samples were obtained by centrifugation after 30 minutes at 4000 rpm. Serum total protein was estimated by Biuret method. Biuret reagent: 4.25 g of potassium sodium tartarate, 1.5 g of cupric sulphate and 2.5 g potassium iodide were dissolved in about 500 mL of distilled water. 4 g of NaOH was dissolved in the solution and made up the volume to 1 L. 0.1 mL aliquots of standard, test plasma and blank (saline) were taken in test tubes. In each test tube 5 mL Biuret reagent was added. Mixed well and kept for 30 minutes. Optical density of test and standard were read against blank at 540 nm by UV spectrophotometer. The protein molecules react with copper sulphate in alkaline medium to give purple color. The concentration of serum total protein was expressed in g/dL.

Statistical analysis
Results were presented as Mean ± SEM. The statistical significance was assessed using unpaired student-‘t’ test by graph pad prism 5 software. A p < 0.05 was considered as statistically significant.

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Table 1: Effect of Trichup Capsule on the body weight of rats during acute toxicity study

<table>
<thead>
<tr>
<th>Single dose</th>
<th>Mean body weight (g)</th>
<th>% body weight gain (Day 0-7)</th>
<th>Mean body weight (g)</th>
<th>% body weight gain (Day 7-14)</th>
<th>% body weight gain (Day 0-14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 mg/kg</td>
<td>179.45 ± 6.42</td>
<td>8.86 ± 4.89</td>
<td>215.48 ± 6.07</td>
<td>10.29 ± 5.11</td>
<td>20.07 ± 5.87</td>
</tr>
<tr>
<td>5000 mg/kg</td>
<td>181.56 ± 6.09</td>
<td>9.08 ± 6.53</td>
<td>218.52 ± 8.28</td>
<td>10.33 ± 6.26</td>
<td>20.35 ± 8.11</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM (n = 6)

Table 2: Effect of Trichup Capsule on visual parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time taken for hair growth initiation (In days)</th>
<th>Time taken for hair growth completion (In days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (NC)</td>
<td>8.83 ± 0.16</td>
<td>28.83 ± 0.60</td>
</tr>
<tr>
<td>Trichup Capsule treated (TED)</td>
<td>7.67 ± 0.45</td>
<td>23.33 ± 0.33</td>
</tr>
<tr>
<td>Trichup Capsule treated (TED × 2)</td>
<td>7.65 ± 0.33</td>
<td>22.67 ± 0.21</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM (n = 6). Where, *p < 0.05 when compared to normal control group

Table 3: Effect of Trichup Capsule on serum total protein

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum total protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (NC)</td>
<td>2.91 ± 0.12</td>
</tr>
<tr>
<td>Trichup Capsule treated (TED)</td>
<td>5.24 ± 0.24</td>
</tr>
<tr>
<td>Trichup Capsule treated (TED × 2)</td>
<td>6.08 ± 0.17</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM (n = 6). Where, *p < 0.05, **p < 0.01 when compared to normal control group
RESULTS

Acute toxicity study
In acute toxicity study, animals did not manifest any signs of toxicity or mortality at both dose level i.e. 2000 mg/kg and 5000 mg/kg. The body weight of rats was found increased after the oral administration of Trichup Capsule. The marked % body weight gain was observed on 7th day and 14th day (Table 1).

Hair growth promoting efficacy study
Treatment of Trichup Capsule at both dose levels showed significant hair growth promoting effect (p < 0.05) on visual parameters in comparison to normal control group (Table 2). As the results shown in Figure 1, rats treated with Trichup Capsule at TED and TED × 2 showed significant hair growth. The length of hair in TED and TED × 2 treated groups were 17.22 mm and 18.50 mm respectively on 30th day, compared to the normal control (13.11 mm). As results presented in Figure 2, hairs diameter were found significantly increased in Trichup Capsule treated group as compared to normal control. TED × 2 group showed more remarkable elevation than TED group (Table 3).

DISCUSSION
Hair loss disorders, although are not life-threatening, are emotionally distressing diseases that make afflicted patients vulnerable. In conventional medicine, though the drug Minoxidil has been reported to be efficacious in stimulating hair growth in Androgenic alopecia, it has also been observed causing various adverse dermatological reactions. Due to the undesirable side-effects, the therapeutic uses of such conventional drugs are being limited. On the other hand, more attention is being paid to herbal medicines which are thought to be exerting their hair promoting activity, with minimal or no side effects or toxicities. Several medicinal plants have been reported in various Ayurvedic literature and so far scientific researches for hair growth promotion, hair vitalizing and anti-oxidant property. However, very few proprietary Ayurvedic oral dosages are available in markets which are considered safe and effective for hair growth. Hence, in the present study, an attempt was made to evaluate the acute toxicity and hair growth promoting activity of a herbo-mineral proprietary Ayurvedic medicine Trichup Capsule, in wistar rats. Results obtained from visual parameters indicate that Trichup Capsule has remarkable effect on promoting hair growth (Table 2). As the selected
test drug contains multiple ingredients it was very difficult to establish exact mechanism of action. However, on basis of earlier investigations it can be postulated that various primary compounds of these ingredients such as flavonoids, polyphenols, triterpenoids and saponins induce hair growth promotion by improving subcutaneous micro-circulation of blood which ensures optimum supply of vital nutrition to the hair follicles. In the present study, length and diameter of hairs were observed significantly increased (Figure 1 and 2). This can be attributed to synergistic effect of all the ingredients of Trichup Capsule. Nutritional factors and serum protein level are also considered affecting the hair growth directly. Treatment of Trichup Capsule significantly increased level of serum total protein. TED × 2 group showed more significant elevation in comparison of TED (Table 3). This suggests that Trichup Capsule is also working on transportation and cellular uptake of nutritional elements into hair follicle and ensures restoration of normal hair growth pattern.

CONCLUSION

On the basis of study data, it can be concluded that Trichup Capsule is safe and having promising hair growth promoting activity which can be attributed to synergistic effect of its multiple ingredients.

ACKNOWLEDGEMENT

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