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EVALUATION OF ACUTE TOXICITY STUDY AND HAIR GROWTH PROMOTING ACTIVITY OF TRICHUP TABLET

Hardik Soni*[1], Ekta Rabadiya [2], Maitreyi Zaveri [2], Sonal Patel [2], Ghanashyam Patel [1]

Vasu Research Centre [1] (A Division of Vasu Healthcare Pvt. Ltd.), 896/A, G.I.D.C., Makarpura, Vadodara - 390010, Gujarat, India

Department of Pharmacognosy and Phytochemistry, K.B. Institute of Pharmaceutical Education and Research [2], Sector - 23, Near GH - 6, Gandhinagar - 382 023, Gujarat, India

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Correspondence to Author:

Hardik Soni

Vasu Research Centre
A division of Vasu Healthcare Pvt. Ltd. 896/A, GIDC, Makarpura, Vadodara – 390010, Gujarat, India

E-mail:hsoni@vasuresearch.com; hardik_ayurved@yahoo.com

ABSTRACT: Hair loss problems can affect physical and mental health of human and have particular relevance during the aging process. In an effort 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 Tablet. 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 Tablet at 198 mg/kg/day and 396 mg/kg/day, p.o., for 30 days respectively. Visual parameters, hair length, hair diameter and serum total protein were determined. The animals did not manifest any signs of toxicity or mortality. Treatment of Trichup Tablet showed significant effect on all visual parameters. Length and diameter of hairs were significantly increased in TED and TED×2. Serum total protein was also observed to be elevated in TED and TED×2 in comparison to normal control. On the basis of study data, it can be concluded that Trichup Tablet is safe and having significant hair growth promoting activity.

INTRODUCTION: Hair is one of the vital parts of body derived from ectoderm of the skin and is one of the protective appendages on the body[1]. Hair growth is a common biological process observed in animals and human beings. Hair loss is an emotionally distressing disease in humans. It is known that various diseases, nutritional deficiency, aging, hormone imbalance, and stress can cause hair loss in both men and women [2, 3].

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To date, the number of patients suffering from hair loss or alopecia has increased dramatically[4]. Many people suffer from hair loss or hair thinning, despite the development of several medical treatments. Therefore, it is important to develop new therapies that prevent hair loss and promote hair growth as well. In this respect, alternative medicine has attracted sufficient interest, although it has not yet been included into mainstream of medical care, due to the limited scientific proofs [5].

Trichup Tablet is a proprietary Ayurvedic formulation which contains extract of *Eclipta alba* (Bhringraj) whole plant [6], *Glycyrrhiza glabra* (Yashtimadhu) root [7], *Emblica officinalis* (Amalaki) fruit [8], *Centella asiatica* (Mandukparni) whole plant [9], *Hibiscus rosa-sinensis* (Japa) flower

[10], *Tinospora cordifolia* (Guduchi) stem [11], *Tribulus terrestris* (Gokshur) fruit [12] and powder of Triphala Churna [13], Shukti Bhasma [14]. It is a proprietary Ayurvedic medicine manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. Majority of ingredients of Trichup Tablet 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 Tablet 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 pelleted 'Sabardan' 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 Tablet was provided by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. The test formulation (Trichup Tablet) 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 Barnes[15].

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 gavages (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 [16].

Hair growth promoting activity: The selected animals were divided in to 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 Tablet) treated group [Therapeutic Effective Dose (TED) – 198 mg/kg/day, p.o., for 30 days]

Group-III (TED×2): Served as test drug (Trichup Tablet) treated group [Double of Therapeutic Effective Dose (TED×2) – 396 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 [17]. Group II and III were treated with Trichup Tablet at 198 mg/kg/day and 396 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 rats under light ether anesthesia from retro orbital plexuses in clean dry centrifuge tubes. Samples were allowed to clot for 30 min at room temperature. Sera from the samples were obtained by centrifugation after 30 min at 4000 rpm. Serum total protein was estimated by Biuret method [18].

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 540nm 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 $p \leq 0.05$ was considered as statistically significant.

RESULTS:

Acute toxicity study: In acute toxicity study, the 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 increased after the oral administration of Trichup Tablet. The marked % body weight gain was observed on 7th day and 14th day **(Table 1).**

TABLE 1: EFFECT OF TRICHUP TABLET ON THE BODY WEIGHT OF RATS DURING ACUTE TOXICITY STUDY

Single dose	Mean body weight (g)		% body weight gain (Day 0-7)	Mean body weight (g)	% body weight gain (Day 7-14)	% body weight gain (Day 0-14)
	0 day	7th day		14th day		
2000 mg/kg	183.67 ± 5.78	199.54 ± 4.82	8.64 ± 5.07	219.23 ± 6.41	9.86 ± 5.72	19.36 ± 6.24
5000 mg/kg	192.78 ± 7.84	210.12 ± 6.74	8.99 ± 6.08	233.10 ± 7.84	10.93 ± 6.57	20.91 ± 7.08

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

Hair growth promoting efficacy study: Treatment of Trichup Tablet at both dose level showed significant hair growth promoting effect ($p<0.05$) on visual parameters in comparison to normal control group **(Table 2).**

TABLE 2: EFFECT OF TRICHUP TABLET ON VISUAL PARAMETERS

Groups	Time taken for hair growth initiation (in days)	Time taken for hair growth completion (In days)
Normal Control (NC)	8.83 ± 0.16	28.83 ± 0.60
Trichup Tablet treated (TED)	7.66 ± 0.42*	24.67 ± 0.21*
Trichup Tablet treated (TED×2)	7.83 ± 0.30*	23.83 ± 0.30*

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

As the results shown in **figure 1,** rats treated with Trichup Tablet at both dose level produced significant ($p<0.01$) hair growth. The length of hair in TED and TED×2 treated groups were 15.61mm and 16.33mm respectively on 30th day, compared to the normal control (13.11mm).

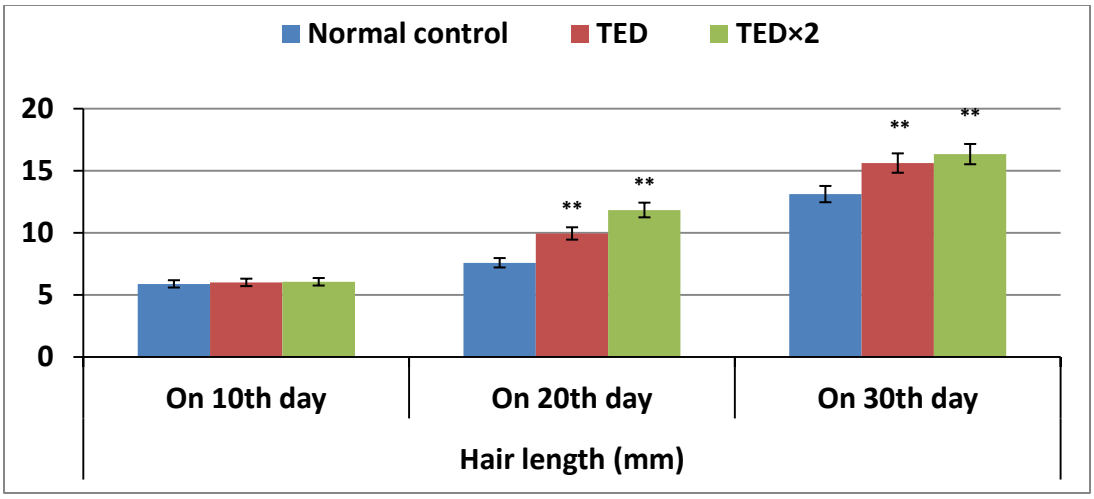

FIGURE 1: EFFECT OF TRICHUP TABLET ON HAIR LENGTH. Values are expressed in Mean ± SEM (n=18). Where, **P<0.01 when compared to normal control group.

As results presented in **figure 2,** hairs diameter were found significantly increased in Trichup Tablet treated group as compared to normal control. TED×2 group (p<0.01) showed more significant effect on diameter of hair than TED group (p<0.05).

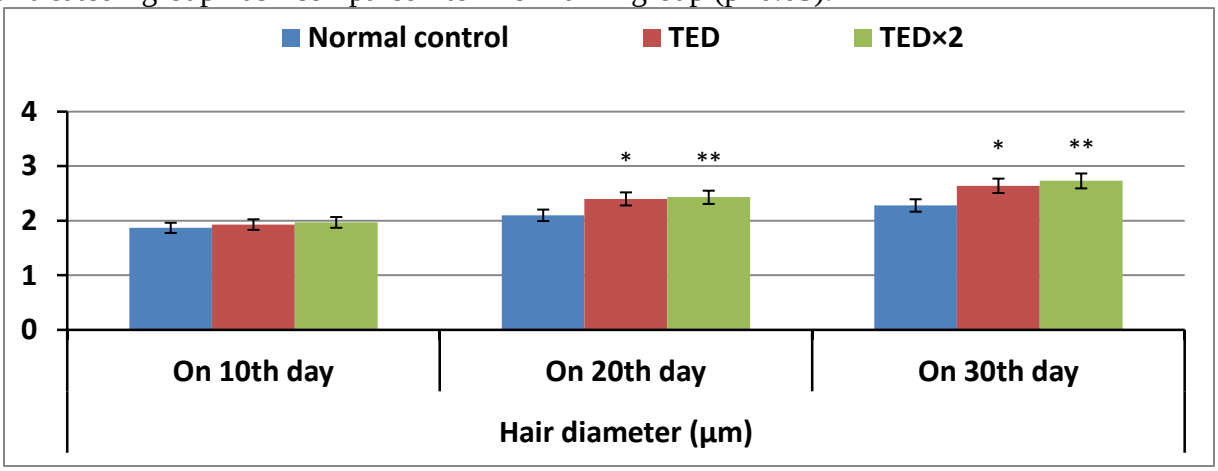

FIGURE 2: EFFECT OF TRICHUP TABLET ON HAIR DIAMETER. Values are expressed in Mean ± SEM (n=18). Where, *p<0.05, **P<0.01 when compared to normal control group.

As shown in Table 3, the level of serum total protein was significantly increased in TED (P<0.05) and TED×2 group (P<0.01) as compared to normal control group. TED×2 group showed more remarkable elevation than TED group **(Table 3).**

TABLE 3: EFFECT OF TRICHUP TABLET ON SERUM TOTAL PROTEIN

Groups	Serum total protein (g/dL)
Normal Control (NC)	2.91 ± 0.12
Trichup Tablet treated (TED)	4.24 ± 0.24*
Trichup Tablet treated (TED×2)	5.82 ± 0.17**

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

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 promoting hair growth in alopecia[19], it has also been observed causing various adverse dermatological reactions, such as pruritis, dryness, scaling, local irritation and dermatitis [20]. 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 Tablet, in wistar rats.

Results obtained from visual parameters indicate that Trichup Tablet has remarkable effect on promoting hair growth (Table 2). As the selected test drug contains multiple ingredients it has been very difficult to establish exact mechanism of action. However, on basis of earlier investigations it can be assumed that various primary compounds of these ingredients such as flavonoids, polyphenols, triterpenoids and saponins exert hair growth promotion by improving subcutaneous micro-circulation of blood which ensures optimum supply of vital nutrition to the hair follicles [6, 18, 21]. In the present study, length and diameter of hairs were observed significantly increased (Figure 1 & 2). This can be attributed to synergistic effect of all the ingredients of Trichup Tablet.

Nutritional factors and serum protein level are also considered affecting the hair growth directly [22]. Treatment of Trichup Tablet significantly increased level of serum total protein. TED×2 group showed more significant elevation in comparison of TED (Table 3). This proves that Trichup Tablet is also working on nutritional element transport at cellular level to ensure optimum hair construction.

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

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