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Effect of *Trigonella foenum-graecum* Linn. (seeds) and *Butea monosperma* Lam. (flowers) on chemotherapy-induced alopecia

Trigonella foenum-graecum Linn. (tohum) ve *Butea monosperma* Lam. (çiçek)'ın kemoterapi ile uyarılan alopesi üzerine etkileri

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SUMMARY

AIM: This study aimed to evaluate the hair growth promoting activity of *Trigonella foenum-graecum* Linn. (seeds) and *Butea monosperma* Lam. (flowers).

METHODS: In the present study, it was investigated to prepare herbal hair ointment formulations of *Trigonella foenum-graecum*, *Butea monosperma* and their polyherbal formulation evaluating the formulations for their hair growth promoting activity, total serum protein level and testosterone level in chemotherapy induced alopecia model.

RESULTS: The formulation showed significant increase in hair length and hair count as compared to control group. The polyherbal formulation helped to reduce the dihydro-testosterone (DHT) level. *Trigonella foenum-graecum* contain riched amount of protein which helped in hair growth and *Butea monosperma* contain riched amount of flavanoid, which has the antioxidant activity by scavenging free radicals and preventing hair fall.

CONCLUSION: The results thus corroborate with the traditionally acclaimed hair growth-promoting capabilities of the plants.

Key words: *Trigonella foenum-graecum*; *Butea monosperma;* Chemotherapy-induced alopecia; Hair-growth activity; Testosterone; Dihydro-testosterone (DHT).

ÖZET

AMAÇ: Bu çalışma, *Trigonella foenum-graecum* Linn. (tohum) and *Butea monosperma* Lam. (çiçek)'nın saç büyümesini artırıcı etkisini değerlendirmeyi amaçladı.

YÖNTEM: Şimdiki çalışmada, *Trigonella foenum-graecum* ve *Butea monosperma*'nın bitkisel saç merhemi formülasyonu ve çoklu bitkisel formülasyonların hazırlanması, kemoterapiyle uyarılan alopesi (kellik) modelinde saç büyümesini geliştiren etkinlikleri, toplam serum protein seviyeleri ve testosterone seviyleri değerlendirilerek araştırıldı.

BULGULAR: Kontrol grubu ile karşılaştırıldığında, formülasyonlar saç boyunda ve saç sayısında belirgin artış gösterdi. Çoklu bitkisel formülasyon dihidrotestosteron (DHT) seviyesini azaltmada yardımcı oldu. *Trigonella foenum-graecum* saç büyümesine yardımcı olan çok miktarda protein içermektedir ve *Butea monosperma*, saç dökülmesini önleyen ve serbest radikal süpürücü aktivitesi ile antioksidan etkinliği olan çok miktarda flavanoid içermektedir.

SONUÇ: Nitekim, sonuçlar bitkilerin geleneksel olarak beğenilen saç büyümesini teşvik edici etkileri ile teyit edilmektedir.

Anahtar kelimeler: *Trigonella foenum-graecum*, *Butea monosperm,* kemoterapi ile oluşturulan kellik, saç büyütücü etkinlik, testosteron, dihidrotestosteron (DHT).

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INTRODUCTION

The three major and frequent toxicities of cytotoxic cancer chemotherapy are bone marrow suppression, gastrointestinal disturbances and alopecia. Alopecia negatively affects a patient's perception of physical appearance, body image, sexuality and self-esteem and deprives patients of the privacy of having cancer. Chemotherapy-induced alopecia (CIA) is considered one of the most negative factors in cancer patient care. The National Coalition for Cancer Survivorship cites CIA as one of the most emotionally upsetting aspects of coping with cancer. Female patients are particularly affected; a survey shows that 47% patients consider CIA the most traumatic side effect of chemotherapy and 8% would reject chemotherapy due to fear of CIA.

Alopecia also results in reduced social interactions in school-age children and teenagers). The negative psychological impact of CIA may have additional undesirable biological consequences, as depression lowers immune function and is associated with cancer progression. Substantial efforts have been expended and consequently, multiple drugs have been developed, to manage chemotherapy-induced bone marrow suppression and gastrointestinal disturbances. In comparison, the development of CIA treatment is lagging and no effective treatments for CIA are available at present [1, 2]. In recent years, many plants have been tested for their hair growth promoting activity viz *Tridax procumbens* [3], *Citrullus colocynthis* [4], *Eclipta alba* [5], *Cuscuta reflexa* [6, 7]. This work was done with an attempt to collect some experimental evidences if the herbs under consideration, which is *Trigonella foenum-graecum* and *Butea monosperma* and their polyherbal formulation are having some potential of preventing hair loss in such cases of chemotherapy-induced alopecia.

MATERIAL AND METHODS

Plant Material

Plant materials of *Trigonella foenum-graecum* Linn. (seeds) and *Butea monosperma* Lam. (flowers) were collected in the month of September-October from the city market of Bhopal. Plants were identified and authenticated in the Department of Pharmacy, Barkatullah University, Bhopal (M.P.). Voucher specimen BUPH-4034 A (*Trigonella foenum-graecum*) and Voucher specimen BUPH-4034 B (*Butea monosperma* Lam.) was deposited.

Preparation of Extract

Approximately 500g of powdered crude drugs were separately extracted with hydro-alcoholic solvent (*Trigonella foenum-graecum* Linn. 50:50, *Butea monosperma* Lam. 70:30) by double maceration process. The macerates were filtered with muslin cloth and concentrated using rotary evaporator to avoid thermal degradation. Phytochemical tests reveals the presence of carbohydrate, alkaloid, flavonoid, steroids, saponins, protein, tannin, amino Acids, cystein and sulphur in *T. foenum-graecum* whereas the hydroalcoholic extract of *B. monosperma* tested positive for carbohydrate, alkaloid, flavonoid, steroids, protein and tannin [8-11].

Ointment Formulation

10% (w/w) hydrophilic ointment USP of hydroalcoholic extracts of Trigonella foenum-graecum, Butea monosperma and their polyherbal were formulated. [12].

Ointment Evaluation

Following parameter used for evaluation of prepared ointment.

a. Primary Skin Irritation test
b. Physical Stability
c. pH

Primary Skin Irritation Test: The skin from the back of six albino rats was shaved on both sides of the back using hair clipper and electric shaver to exposed test areas. On cleaned test sites, the prepared formulations were applied and visual observation was made for the appearance of any irritation or erythema for a total period of 72 hr. after the application of test preparations [13].

Physical Stability: The prepared formulations were stored at room temperature and at elevated temperature 40±2°C for 30 days. After 15 and 30 days, the formulation was tested for its physical characteristics like colour and odour [13].

pH: The pH of prepared ointment was evaluated by pH meter [13].

Animals

For evaluating the hair-growth potential using chemotherapy-induced alopecia model, albino rats weighing 120-150g were maintained at standard conditions, fed with standard diet ad labitium and allowed free access to drinking water for one week.

Hair-Growth Activity

The model has been used with certain modifications in the protocol already published in

scientific literature. All 24 albino rats of both sex male and female were depilated with the help of wax (2.5 cm2 area) and rest for 14 days for anagen induction. After 14 days of depilation administered cyclophosphamide (120 mg/kg, i.p.) to all albino rats in divided dose of 40 mg/kg/day for 3 days. The albino rats were then maintained resting for 9 days after the administration of cyclophoshamide and hair fall was then investigated. Albino rats were then divided in four groups. Each group contains six albino rats. On 24th day of depilation, in Group 1 received Ointment base, Group 2 received 10% w/w ointment of hydroalcoholic extracts of Trigonella foenum-graecum, Group 3 received 10% w/w ointment of hydroalcoholic extracts of *Butea monosperma* and Group 4 received total 10% w/w ointment of Polyherbal hydroalcoholic extracts of *Trigonella foenum-graecum* and *Butea monosperma*, for next 21 days, once daily. On 45th day of depilation all samples were collected [2]. The blood samples were collected from retro-orbital plexus for biochemical parameter, Skin was collected (from depilated area) for histological investigation and hair was also collected and its physical characteristics studied.

Hair length: Hair was plucked randomly from the each group on 45th day of the treatment from depilated area (where hair fall patches observed) with the help of clipper and measured the hair length of each rat with the help vernier caliper and the mean of hair length was calculated [14].

Hair density: A hole of 1cm^2 was made on card board. Then the card board set on the desired depilated area (where hair fall patches observed) on the back of rat after 45 days of depilation. The hair was trimmed of desired depilated area and the hair was cut with the seizure. The hair was manually counted. [15].

Total Protein Estimation

Total serum protein in blood estimated by Modified Biuret method. Procedure:

Name of reagent	Blank (µl)	Standard (µl)	Test (µl)
Serum	-	-	10
Reagent 1	1000	1000	1000
Reagent 2	-	10	-

Above reagent mixed well and incubate at 37°C for 5 min. The analyzer was set on blank with the help of reagent blank. The absorbance of the std. was measured by Jasco V-530 Germany double beam spectrophotometer followed by the test. Result was calculated with the help of given formula [16].

$$\text{Total protein conc. (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of std.}} \times 6.5$$

Testosterone Level

Procedure: The blood sample of each rat was collected by retro-orbital plexus method and send to Dr. Lal Pathology labs Pvt. Ltd. for estimation of blood testosterone level by immunoassay method [17, 18].

During the irritation test none of the prepared formulations showed any erythema or edema, indicating that the prepared formulation were non irritant on the skin of rat. The pH of the all formulations was found between 6.5 to 7.5 the stability of ointment observed on 15th day and 30th day of preparation at room temperature and 40±2°C. In ointment, there was no change observed in colour and odour. Following parameters like hair length, hair density (Table 1), total serum protein, testosterone level (Table 2) and hair follicle in anagen phase (Table 3) show significant effect in polyherbal hydroalcoholic extracts of *Trigonella foenum-graecum* and *Butea monosperma*. The polyherbal formulation showed a maximum hair growth potential when compared to single drug extract. Thus, the drugs under investigation helped in hair growth and protect against chemotherapy-induced alopecia.

Statistical Analysis

The results were expressed as mean±standard deviation (SD) (n=3) and P< 0.05 was considered statistically significant. Statistical analysis was performed with SPSS 10.1 for Windows$^{@}$ (SPSS$^{@}$, Chicago, IL, USA). Student t-test was performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diago, California, USA.

DISCUSSIONS

In *Trigonella foenum-graecum* have good Phenolic content and also have good reducing power. The fenugreek contain significant amount of protein content and different type of amino acids which may help in hair growth (both length and density).

Table 1. Effect of different ointment formulation on hair growth activity (Hair Length and Hair Density) on albino rats in chemotherapy induced alopecia model.

S. No.	Groups	Drug	Formulation	Hair Length (mm) (mean±sd)	Hair density (per cm^2) (mean±sd)
1.	Group 1	Vehicle	Ointment Base	1.98±0.109	1242.5±38.69
2.	Group 2	Hydroalcoholic Extract of *TFG*	Ointment (10%w/w)	3.10±0.17	1892±34.6
3.	Group 3	Hydroalcoholic Extract of *BM*	Ointment (10%w/w)	3.26±0.15	1938.66±34.30
4.	Group 4	Polyherbal Extract	Ointment (10%w/w)	3.43±0.16*	2018.33±29.94*

Grp-1: Control, Grp-2: Trigonella foenum-graecum, Grp-3: Butea monosperma, Grp-4: Polyherbal hydroalcoholic extracts of Trigonella foenum-graecum and Butea monosperma
*Significance (P value < 0.01) as campared of control group to to all 3 test group.

Table 2. Effect of different ointment formulation on hair growth activity (Total serum protein level g/dl and Testosterone level ng/ml) on albino rats in CIA model

S. No.	Groups	Drug	Formulation	Total serum protein (g/dl) (mean±sd)	Testosterone (ng/ml) (mean±sd)
1.	Group 1	Vehicle	Ointment Base	5.89±0.10	1.265±0.03
2.	Group 2	Hydroalcoholic Extract of *TFG*	Ointment (10% w/w)	6.82±0.08*	1.24±0.014
3.	Group 3	Hydroalcoholic Extract of *BM*	Ointment (10% w/w)	6.53±0.09	1.21±0.02
4.	Group 4	Polyherbal Extract	Ointment (10% w/w)	6.63±0.055	1.20±0.021*

Grp-1: Control, Grp-2: *Trigonella foenum-graecum*, Grp-3: *Butea monosperma*, Grp-4: Polyherbal hydroalcoholic extracts of *Trigonella foenum-graecum* and *Butea monosperma*
*Significance (P value < 0.01) as compared of control group to to all 3 test group.

Table 3. Effect of different ointment formulation on hair growth activity (No. of hair follicles in different stages) of albino rats in chemotherapy induced model

S. No	Groups	Drug	Percentage of hair follicles		
			Topical		
			Anagen	Catgen	Telogen
1.	Group 1	Vehicle	50.64	1.83	47.5
2.	Group 2	Hydroalcoholic Extract of *TFG*	62	1.5	36.5
3.	Group 3	Hydroalcoholic Extract of *BM*	64.16	1.166	34.66
4.	Group 4	Polyherbal Extract	66.03	1	32.97

Grp-1: Control, Grp-2: *Trigonella foenum-graecum*, Grp-3: *Butea monosperma*, Grp-4: Polyherbal hydroalcoholic extracts of *Trigonella foenum-graecum* and *Butea monosperma*

The alkaloid (Trigonelline) present in fenugreek it have resemble structure to nicotinic acid so it may show CNS stimulant activity by increasing blood flow and help in hair growth. In *Butea monosperma*, the high level of phenolic content could also have a significant reducing power. The antioxidant activity may help in hair growth by increasing the cell proliferation in dermal papilla and it scavenges the free radicals.This suggests and prevents the hair fall. It decreases the DHT level in blood. When DHT becomes more active, it reacts with sebum and cholesterol found on and within the scalp. This DHT/sebum/cholesterol mix is shed into hair follicle, where it slowly narrow the tiny opening, causing the follicle itself to become shorter and shallower within the scalp. The Butea monosperma helped to maintain the anagen phase of hair for long time and reduce the telogen phase. Polyherbal formulation of Trigonella foenum-graecum and Butea monosperma showed significant hair growth as compare to single drug extract. So it maybe has any synergism effect.

CONCLUSION

The polyherbal extract of *Trigonella foenum-graecum* and *Butea monosperma* showed significant hair growth as compared to single drug extract. In conclusion both drug have synergistic effect and suggest for combine use in future for hair growth formulation. Further studies on both single drug and polyherbal formulation require for determination of which chemical constituent responsible for hair growth and which combination of chemical constituent show synergism effect. Lastly we can predict with the above findings that the formulation is promising and even better results are expected with variation in the proportion of these drugs.

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