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Impact of *Trigonella foenum-graecum* Leaves Extract on Mice Hair Growth

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ABSTRACT

The aim of present study was to investigate hair growth promoting effects of extracts of *Trigonella foenum-graecum* leaves. The extracts of powdered leaves were obtained in various solvents (petroleum ether, chloroform, methanol, ethanol and distilled water) using hot and cold extraction methods. Phytochemical analysis of leaves was performed on powder and extracts of leaves using already reported methods. Mineral analysis was done using atomic absorption spectrophotometer. Hair growth promoting effects were examined using alopecia mouse model. Phytochemical analysis demonstrated the presence of higherquantities of carbohydrates, proteins and secondary metabolites in methanol compared to other extracting solvents. Moreover, only ethanol (5 and 10%) and petroleum ether (5%) demonstrated significant hair growth promoting effects ($p < 0.05$) compared to standard, *i.e.,* 5% minoxidil and extracts in other solvents. Likewise, ethanol (5% and 10 %) and petroleum ether (5%) extract had significant impact ($p < 0.05$) on hair length in comparison to minoxidil, however, no significant differences were observed between ethanol (5% and 10%) extracts and minoxidil on mice hair diameter. Taken together, our data suggested that ethanol extracts of leaves of *T. foenum-graecum* had significantly higher growth promoting effects compared to standard, *i.e.,* minoxidil. Thus, results from our study set the stage of further studies to identify the constituents in ethanol extracts, responsible for hair growth promoting effects.

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MI and HS design the study. FI and MA conducted the experiments and FI, HS and ZS analyzed data. HS and BS wrote the article while MI and ZS edited it.

Key words
Fenugreek, Hair growth, Hair treatment, Trigonella foenum-graecum, Alopecia, Zinc.

INTRODUCTION

Thehair, a vital part of human body, not only protects scalp against detrimental effects of the environment but also plays an important role in garnishing one's personality. They are derived from upper layer of the skin and are evolved from epidermis of the embryo (Kaushik *et al*., 2011). Hair growth is a complex process involving extremely controlled cycles, *i.e.,* hair generation, prolongation and shedding, however, the exact process is still unclear. It is estimated that approximately 85-90% hairs on the scalp are in synthesis phase while the remaining exist in shedding phase (Schulz *et al*., 2006). Several synthetic hair tonics are available that promote hair growth, yet with considerable side effects when used for a longer period of time (Wijaya *et al*., 2013).

In this context, *Trigonella foenum-graecum,* belonging to family Fabaceae, has been used traditionally for various pharmacological effects, such as anti-diabetic, anti-cancer, anti-fungal, anti-pyretic, anti-bacterial and anti-oxidant (Yadav and Kaushik, 2011). Besides, the seeds of *T. foenum-graecum* have been used as anti-lice, anti-dandruff and for hair growth and soothing effects (Didarshetaban *et al*., 2013; Ziyyat *et al*., 1997).

Currently, minoxidil, a synthetic hair tonic and a vasodilator, is available in the market in various concentrations and is used for hair growth promoting effects. It increases the blood circulation to the scalp, subsequently increases the hair growth.In first year thehair growth is at its peak, which decreases in subsequent years along with some side effects, like burning, itching and soreness of eyes (Purwal *et al*., 2008). Studies have shown that natural sources are more reliable than Minoxidil, for example, in male albino rats the use of *Eclipta alba* resulted in enhanced hair growth activity than minoxidil (Roy *et al*., 2008). Likewise, *Citrullus colocynthis* also demonstrated improved hair growth promoting effects than the minoxidil (Roy *et al*., 2007). It has been documented that treatment effects with synthetic drugs are limited and transient, and may cause toxicity on prolong usagethus advocating the use of natural products for medical purpose (Paus, 2006). However, despite numerous reports regarding hair growth promoting effects of natural compounds, minoxidil is still considered the gold standard synthetic hair tonic utilized

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for hair growth effects.

According to literature evidence, the hair growth promoting effects of seeds of *T. foenum-graecum* were comparable with minoxidil (Wijaya *et al*., 2013). However, no literature evidences exist that utilized *T. foenum-graecum* leaves in hair growth promotion studies. Thus, we aimed at comparing the hair growth promoting effects of extracts of *T. foenum-graecum* leaves with that of minoxidil using mice alopecia model.

MATERIAL AND METHODS

Plant material and chemicals

T. foenum-graecum plant, 20 kg, was purchased from local market of Lahore, Pakistan. Leaves of the plant were dried, crushedpulverized and stored in air tight containers. Chemicals like solvents were purchased from BDH, England, while quercetin and gallic acid were procured from Sigma Life Sciences and Sinochem, respectively. All other chemicals were procured from Merck, Germany. Triton X and Folin and Ciocalteu's reagents were purchased from Uni-Chemicals, Ireland. Standard solutions of different metals were purchased from Acros Organics, USA, and minoxidil 5% were purchased form Brookes Pharma Private Limited, Pakistan.

Extraction from leaves of T. foenum-graecum

Extraction was carried out using hot and cold method (Handa *et al*., 2008). Briefly, two fifty grams powdered material was sequentially extracted using solvents; petroleum ether, chloroform and methanol, and twenty five grams powdered material was extracted in ethanol and distilled water employinghot and cold extraction methods, respectively.

Phytochemical analysis of leaves extract

Phytochemical analysis was done to identify chemical compounds present in the leaves of *T. foenum-graecum*.

Primary metabolites

Total proteins were determined using a method described by Lowry *et al*. (1951). Briefly, after mixing with distilled water and Triton-X, reagent C, reagent A (2% Na_2CO_3 in 0.1 N NaOH), reagent B (0.5 % $CuSO_4$ in 1% potassium sodium tartrate) and folin-ciocalteau's were mixed. Later, absorbance was measured at 600nm against the blank, using bovine serum albumin (BSA) as standard.

Total lipids were estimated by extraction in n-hexane and by subtracting weight of dried material from the weight of a round bottom flask (Besbes *et al*., 2004).

Secondary metabolites

Total polysaccharides were determined according to the protocol described previously (Hussain *et al*., 2008). Briefly, extract was mixed with 80% hot ethanol, centrifuged and mixed with anthrone reagent. Final residue was dried and extracted in 1:1 (v/v) mixture of 25 % HCl and water, centrifuged and supernatant was again mixed with distilled water and 4 ml of anthrone reagent followed by absorbance measurement at 630 nm against the blank. Glucose solution was used as standard solution.

Total glycosaponins were estimated according to protocol described previously (Hussain *et al*., 2008). Briefly, extract was refluxed with methanol for 30 min andprecipitated in a tarred beaker containing 50 ml of acetone. Total glycosapoins were calculated using formula:

Total glycosaponins (%) = weight of precipitate / weight of sample x 100

Total polyphenols were determined by using formula described by Slinkard and Singleton (1977). Briefly, dilutions of gallic acid and extract solution (200µL) were prepared and were added to 200µL of Folin–Ciocalteau's reagent and 1 ml of 15 % $Na_2CO_3$to make the volume upto 3ml with methanol. After incubation for 90 min at room temperature, absorbance was measured at 760 nm against the blank.

For flavonoids estimation, quercetin was used as standard. Briefly, 200 µg/ml of each quercetin and extract solution were added to methanol to make the final volume of 1 mL followed by the addition of 100µl of 1M potassium acetate, 100 µl of 10% aluminium nitrate and 4.6 ml distilled water. Solutions were incubated for 45 min at room temperature followed by absorbance measurement at 415 nm against blank (Chang *et al*., 2002).

Mineral estimation

Mineral estimation procedure was carried out using method described by Ahmad *et al*. (2014). Estimation of calcium, iron, zinc, magnesium and potassium was performed using atomic absorption spectrophotometer.

Study animals

Thirty-two male albino mice weighing 20±10 g were purchased from University of Veterinary and Animal Sciences, Lahore, Pakistan. Mice were acclimatized for seven days in University College of Pharmacy animal housing facility under 12 h dark light cycle on standard chow. The study protocol was approved by Animal Research Ethical Committee, Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan, reference No. AEC/UCP/1040/4313.

Hair tonic formulation and evaluation

The components and concentrations of hair tonic formulations are described in Table I. All the extracts of *T.*

foenum-graecum leaves were diluted with distilled water and later homogenized with 98% ethanol using ultra sonic mixer. Finally butylene glycol was added to hair tonic (Wijaya *et al*., 2013). Evaluation of hair tonic was done through organoleptic test, homogeneity test and pH test.

Studies on hair growth promoting effects of extracts

Mice were randomly segregated into eight groups, four in each, *i.e.,* positive control group; treated with 5% minoxidil solution, negative control; vehicle only, untreated and test groups. Extracts were used in two different concentrations; 5% and 10%. Mice were shaved on the dorsal side using hair removing cream (VEET® cream) and were kept for 24 h before applying treatments for any untoward reactions. Thereafter, 100µl of test samples were applied on designated area, left and right dorsal side, and day one was considered as zero time point (Wijaya *et al*., 2013).

Qualitative evaluation of hair growth

The qualitative evaluation was done by visual comparison of time taken to cover the shaved area in a stipulated time that is 21 days (Adhirajan *et al*., 2003).

Hair length measurements

Ten hair strands were randomly plucked from each side of dorsal area on day 7, 14 and 21. Hair was straightened out and length was measured using digital vernier caliper as described previously (Adhirajan *et al*., 2003).

Measurement of hair diameter

Hair diameter was measured using microscope with ocular micrometer as described previously (Adhirajan *et al*., 2003). The data was presented as an average hair diameter, representative of three biological replicates.

RESULTS

Metabolites and minerals of leaves of T. foenum-graecum

The percentage contents of primary and secondary metabolites in the leaves of *T. foenum-graecum* are summarized in Table II. The leaves of *T. foenum-graecum* were found to be rich in carbohydrates (52.31 % ± 2.46), proteins (18.54% ± 0.80) and lipids (5.443% ± 0.07) (Table II). Among all the extracts, methanol extract demonstrated maximum concentration of all the secondary metabolites, including total polysaccharides (30.54% ± 0.06), total polyphenols (107.35% ± 0.20), total flavonoids (14.25% ± 0.06) and total glycosaponins (88.73% ± 0.15) (Table II). The ethanol extract contained 28.74% total polysaccharides, 105.86% total polyphenols, 13.70% total flavonoids and 74.4% total glycosaponins (Table II).

Table I.- Components and concentrations of different hair tonic formulation.

Ingredient	Hair tonic formulation												
	Positive control	Negative control (*%)	Untreated	Petroleum ether (%)		Chloroform (%)		Methanol (%)		Ethanol (%)		Aqueous (%)	
Extract	-	-	-	5	10	5	10	5	10	5	10	5	10
Minoxidil	5	-	-	-	-	-	-	-	-	-	-	-	-
Butylene glycol	10	10	-	10	10	10	10	10	10	10	10	10	10
Ethanol 98%	25	25	-	25	25	25	25	25	25	25	25	25	25
Distilled water	60	65	-	60	55	60	55	60	55	60	55	60	55

*, w/w.

Table II.- Estimation of primary and secondary metabolites of powdered leaves of *Trigonella foenum-graecum*.

Sample powder	Primary metabolites		
	Total proteins (% contents ± SD)	Total lipids (% contents ± SD)	Total carbohydrates (% contents ± SD)
	18.54 ± 0.80	5.443 ± 0.07	52.31 ± 2.46
Sample extracts	**Secondary Metabolites**		
	Total polysaccharides (% contents ± SD)	Total polyphenols (% contents ± SD)	Total flavonoids (% contents ± SD)	Total glyco-saponins (% contents ± SD)
Petroleum ether	19.99 ± 0.10	90.74 ± 2.96	4.66 ± 0.09	34.76 ± 0.05
Chloroform	21.74 ± 0.06	91.43 ± 0.13	11.20 ± 0.09	52.2 ± 0.20
Methanol	30.54 ± 0.06	107.35 ± 0.20	14.25 ± 0.06	88.73 ± 0.15
Ethanol	28.74 ± 0.06	105.86 ± 0.46	13.70 ± 0.18	74.4 ± 0.30
Water	17.77 ± 0.08	102.76 ± 2.29	4.26 ± 0.12	59.83 ± 0.25

While, the secondary metabolites in petroleum ether extract, include19.99% total polysaccharides, 90.74% total polyphenols, 4.66% total flavonoids and 74.4% total glycosaponins (Table II). In aqueous and chloroform extract the concentration of secondary metabolites were as follows; 17.77% and 21.74% of total polysaccharides, 102.76% and 91.43% of total polyphenols, 4.26% and 11.2% of total flavonoids and 59.83% and 52.2% of total glycosaponins, respectively (Table II). We also found considerable concentration of calcium (0.80 mg/g), magnesium (5.79 mg/g), potassium (1.42 mg/g), iron (7.66 mg/g), and zinc (2.32mg/g) using atomic absorption spectrophotometer in sample of leaves of *T. foenum-graecum* (Table III).

Characteristics of hair tonic

Organoleptic evaluation of hair tonics resulted in pungent smell while aqueous extract hair tonic had rotten egg smell. There was difference in color of different extracts hair tonic (Table IV). Homogeneity test showed that all hair tonics were homogenous and were smoothly spreading on skin. The pH test results are summarized in Table IV. The pH of minoxidil hair tonic was 6.11. The acidity of all hair tonics were within the pH range of a normal human skin, *i.e.*, 4.5-6.5 (Tranggono, 2007).

Table III. Mineral analysis of powder of leaves of *Trigonella foenum-graecum.*

Element	Concentration (mg/g)	Element	Concentration (mg/g)
Iron	7.6	Calcium	0.8
Magnesium	5.7	Zinc	2.3
Potassium	1.4		

Table IV.- Organoleptic evaluation of fenugreek hair tonic.

Formulations		Color	pH
Petroleum ether	5%	Light brown	4.78
	10%	Brown	5.09
Chloroform	5%	Light brown	4.77
	10%	Brown	5.42
Methanol	5%	Light reddish brown	5.83
	10%	Dark reddish brown	6.09
Ethanol	5%	Yellowish brown	6.19
	10%	Brown	6.27
Aqueous	5%	Green	6.21
	10%	Blackish green	6.45

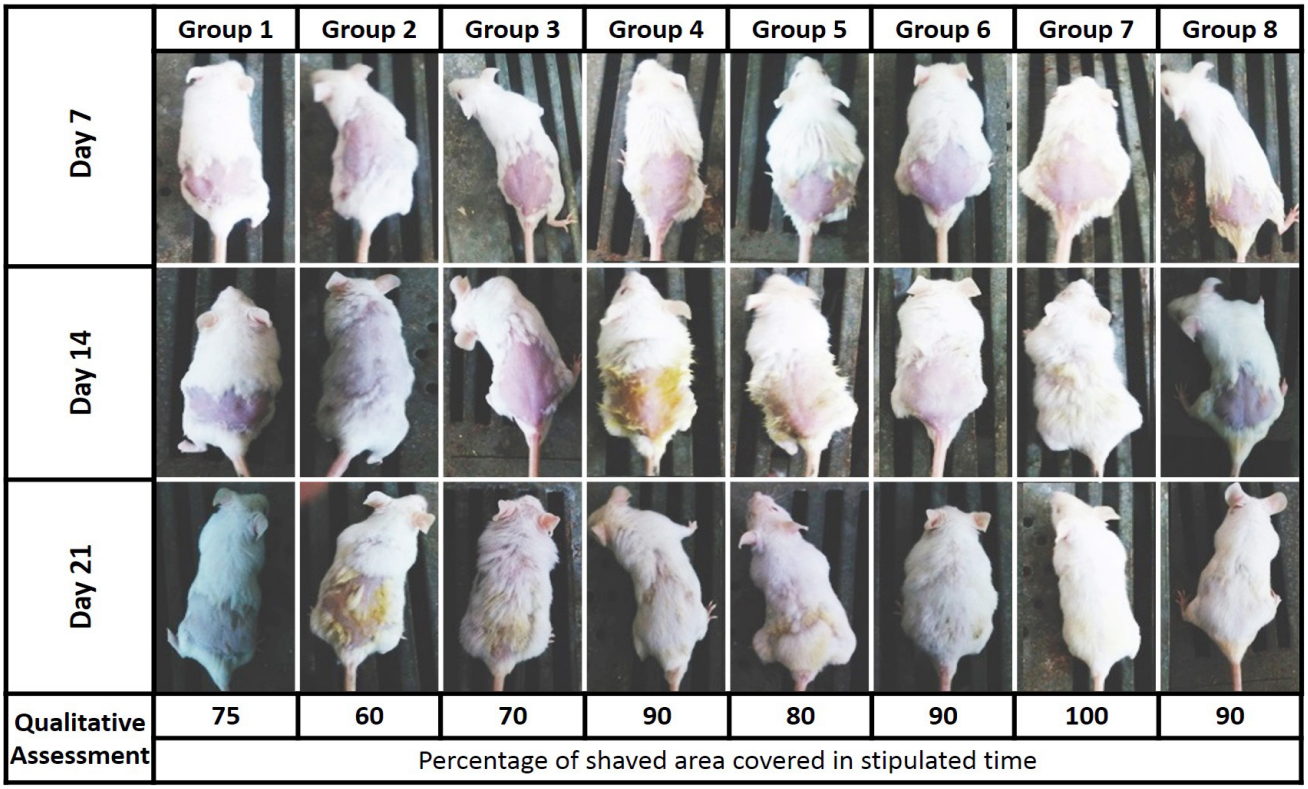

Fig. 1. Qualitative assessment of hair growth effects of various extracts of leaves of *Trigonella foenum-graecum.*

Hair growth promoting effects of leaves extracts

Mice representing each group and at different time points (Day 7, 14 and 21) are shown in Figure 1. Data suggested that ethanol extract, group 7, demonstrated maximum hair growth promoting effects not only when compared with standard (group 1) but also compared to all other groups having different solvents (Fig. 1).

Qualitative evaluation of hair growth on different groups is given in Figure I. Percentage of shaved area covered with hairs in all the groups with in a stipulated timeof 21 days was as follows; group 1, 75%; group 2, 60%; group 3, 70%; Group 4, 90%; group 5, 80%; group 6, 90%; group 8, 90% and only group 7 demonstrated 100% hair coverage with in a stipulated time of 21 days (Fig. 1).

Table V.- Effect of fenugreek leaves extract on mice hair length.

Test groups	Treatment	Average length (mm) ± SD		
		7th day	14th day	21st day
Group 1	P control	3.81 ± 0.38	4.65 ± 0.86	6.96 ± 0.72
Group 2	N control	3.81 ± 1.90	4.17 ± 0.68	6.10 ± 1.11
Group 3	Untreated	2.54 ± 0.27	3.90 ± 0.26	5.17 ± 0.61
Group 4 Petroleum ether	5%	3.80 ± 0.64	6.64 ± 1.54	8.04 ± 0.38
	p – values	0.98	0.08	0.03*
	10%	4.80 ± 1.20	5.94 ± 0.51	7.44 ± 1.31
	p – values	0.24	0.07	0.54
Group 5 Chloroform	5%	4.38 ± 0.61	5.46 ± 0.91	6.96 ± 0.53
	p – values	0.24	0.28	0.98
	10%	4.50 ± 0.43	5.65 ± 0.86	6.75 ± 0.56
	p – values	0.62	0.19	0.69
Group 6 Methanol	5%	4.09 ± 0.64	4.73 ± 0.10	8.40 ± 0.86
	p – values	0.55	0.88	0.06
	10%	3.85 ± 0.06	4.83 ± 0.34	7.85 ± 0.33
	p – values	0.88	0.75	0.11
Group 7 Ethanol	5%	5.81 ± 1.62	7.44 ± 1.55	8.52 ± 1.02
	p – values	0.09	0.01*	0.04*
	10%	6.19 ± 0.50	7.79 ± 1.51	8.86 ± 1.40
	p – values	0.001**	0.02*	0.04*
Group 8 Aqueous	5%	5.15 ± 0.47	5.32 ± 0.37	6.87 ± 0.83
	p – values	0.01*	0.27	0.89
	10%	4.85 ± 0.76	5.61 ± 0.88	7.39 ± 0.57
	p – values	0.10	0.21	0.51

p values were obtained by comparing with test groups to positive control; *, p value < 0.05 – 0.01; **, p value < 0.0009 – 0.001; P control, positive control; N control, negative control.

Data on hair length measurements are summarized in Table V. At day 7, standard and petroleum ether (5%) groups demonstrated similar hair growth patterns, while only ethanol 10% (6.19±0.50 mm, p=0.001) and aqueous 5% extracts (5.15±0.47 mm, p=0.01) exhibited significantly higher hair growth effects on hair length in comparison to standard (3.81±0.38 mm) and petroleum ether (Table V). Likewise, in the second week (day 14), ethanol group (5 and 10%) showed significantly higher growth promoting effects (5%; 7.44±1.55 mm, p=0.01, 10%; 7.79 ± 1.51 mm, p=.0.02) compared to positive and all other test groups, including group 4; petroleum ether 5% (6.64 ± 1.54 mm) and petroleum ether 10% (5.94 ± 0.51 mm), group 5; chloroform 5% (5.46 ± 0.91 mm), chloroform 10% (5.65 ± 0.86 mm), group 6; methanol 5% (4.73 ± 0.10 mm) and methanol 10% (4.83 ± 0.34 mm) and group 8; aqueous 5% (5.32 ± 0.37 mm) and aqueous 10% (5.61 ± 0.88 mm) (Table V). On the 21st day petroleum ether 5% (8.04 ± 0.38 mm, *p= 0.03*) along with ethanol, 5% (8.52 ± 1.02 mm, *p=0.04)* and 10 % (8.86 ± 1.40 mm, *p= 0.04*), exhibited highly significant results in comparison to positive group (6.96 ± 0.72 mm) (Table V).

Table VI.- Effect of fenugreek leaves extract on mice hair diameter.

Test groups	Treatment	Average diameter (µm) ± SD		
		7th day	14th day	21st day
Group 1	P control	5.00 ± 0.41	5.20 ± 1.29	7.29 ± 0.86
Group 2	N control	5.41 ± 0.34	5.83 ± 0.41	6.66 ± 0.22
Group 3	Untreated	5.00 ± 0.33	5.27 ± 0.63	6.80 ± 0.24
Group 4 Petroleum ether	5%	4.69 ± 0.59	5.00 ± 1.22	6.38 ± 0.48
	p – values	0.50	0.82	0.46
	10%	5.52 ± 0.62	6.25 ± 0.58	6.25 ± 0.58
	p – values	5.83	0.35	0.21
Group 5 Chloroform	5%	4.30 ± 0.24	4.86 ± 1.04	4.86 ± 0.86
	p – values	0.06	0.72	0.01*
	10%	4.86 ± 0.63	5.20 ± 0.88	6.11 ± 0.48
	p – values	0.76	1.0	0.09
Group 6 Methanol	5%	4.58 ± 0.41	4.72 ± 0.24	5.97 ± 1.57
	p – values	0.28	0.55	0.21
	10%	5.00 ± 1.44	5.13 ± 0.63	5.62 ± 1.47
	p – values	1.0	0.93	0.14
Group 7 Ethanol	5%	5.10 ± 0.52	5.83 ± 1.17	6.25 ± 1.44
	p – values	0.78	0.50	0.26
	10%	5.41 ± 1.44	5.52 ± 0.62	7.29 ± 0.24
	p – values	0.65	0.67	1.0
Group 8 Aqueous	5%	5.13 ± 0.63	5.69 ± 2.29	6.66 ± 2.35
	p – values	0.76	0.73	0.63
	10%	5.00 ± 0.83	5.41 ± 2.53	6.25 ± 1.17
	p – values	1.0	0.89	0.27

p values were obtained by comparing with test groups to positive control; *, p value < 0.05 – 0.01; P control, positive control; N control, negative control.

Comparison of hair diameter after ethanol and minoxidil treatment

Next we evaluated the impact of different extracts on hair strand diameter in comparison to standard and controls. As shown in Table VI, at day 7 no significant difference between group 1 (5.00 ± 0.41 µm), group 2(5.41 ± 0.34 µm) and group 3 (5.00 ± 0.33 µm) were observed, while comparison of these groups with other groups showed significant results except for extracts formulated in 10 % methanol (5.00 ± 1.44 µm), ethanol (5.41 ± 1.44 µm) and aqueous base (5.00 ± 0.83 µm) (Table VI). On Day 14, no significant differences were observed between group 1 (5.20 ± 1.29 µm), group 2 (5.83 ± 0.41 µm) and group 3 (5.27 ± 0.63 µm), and also with rest of the groups (Table VI). On 21st day, group 1 (7.29 ± 0.86 µm) demonstrated significant differences in mouse hair diameter compared to group 2 (6.66 ± 0.22 µm), group 3 (6.80 ± 0.24 µm) and group 5 in 5% chloroform (4.86 ± 0.86 µm, p=0.01) only, but not with 10% chloroform extract. However, no significant differences in hair diameter were observed when all other groups were compared with group 1, group 2 and group 3 (Table VI).

DISCUSSION

It has already been demonstrated in a case study involving human volunteers that the oral intake of seeds of *T. foenum-graecum* as a food supplement can increase hair growth in these volunteers (Schulz *et al*., 2006). Similarly, the seeds of *T. foenum-graecum* have been shown to improve the hair growth in mice alopecia model (Wijaya *et al*., 2013). Interestingly, use of ointment, containing seeds of *T. foenum-graecum,* demonstrated positive hair growth promoting effects in chemotherapy induced alopecia (Gupta *et al*., 2013). In the present study we found that in a stipulated time of 21 days, extracts made from the leaves of *T. foenum-graecum*; ethanol (5% and 10%) and petroleum ether 5%, exhibited significant hair growth promoting effects in alopecia mouse model. Data further revealed that the leaves of *T. foenum-graecum* are rich source of carbohydrates, proteins, glycosaponins, known to possess anti-diabetic, anti-hypercholeterolemic, hepatoprotective and anti-cancer effects (Laila *et al*., 2014; Manivannan *et al*., 2013), flavinoids and polyphenols, known to have anti-oxidants properties and protect against cancer, aging and cardiovascular diseases (Huang *et al*., 2009; Pandey and Rizvi, 2009). Interestingly, all these metabolites were favorably present in methanol extract compared to other extracting solvents.

Seemingly, the beneficial effects of *T. foenum-graecum* against cancer and aging related infections may be attributable to the presence of flavonoids known to act against various diseases caused by viruses, bacteria, fungus, they protects against oxidation, inflammation and cancer (Nazni and Dharmaligam, 2014; Priya *et al*., 2011; Tanwar and Modgil, 2012). Additionally, we found quantifiable concentrations of several minerals in the extracts of *T. foenum-graecum* as leaves, such as iron, magnesium, potassium, calcium and zinc. Several lines of evidences suggest a strong relationship between vitamins, minerals and other phytonutrients with cancer and their protection against cancer (Ghiringhelli *et al*., 2012; Nazni and Dharmaligam, 2014; Priya *et al*., 2011; Tanwar and Modgil, 2012). Likewise, potassium plays a pivotal role in regulating arrhythmias, hypertension, while its deficiency leads to many problems in human body (Ekinci *et al*., 2004). Similarly, calcium can be helpful in combating heart diseases, cancer and prevents osteoporosis (Khan *et al*., 2011). Interestingly, deficiency of zinc has been shown to cause hair loss, growth retardation, delays in wound healing and psychiatric problems (Khan *et al*., 2011).

Despite documented evidences of positive effects of *T. foenum-graecum* on hair growth, the exact mechanism is still obscure (Schulz *et al*., 2006). We found that among all the extracts and compared to minoxidil, only ethanol and petroleum ether extracts of leaves of *T. foenum-graecum* demonstrated significant effects on mice hair growth and length, which may be due to rich phenolic contents having reducing power, anti-oxidant activity and free radical scavenging properties, protecting against genotoxic insults, thus rejuvenating the cells to sustain cell proliferation (Gupta *et al*., 2013). Anti-oxidant effects of leaves of *T. foenum-graecum* have been demonstrated in various models, such as drug induced hepatotoxicity goat model and streptozotocin induced diabetic mouse (Annida and Prince, 2005; Devatkal *et al*., 2012; Meera *et al*., 2009). Another potential constituent that might contribute towards hair growth promoting effects of extracts of leaves of *T. foenum-graecum,* could be the presence of considerable amount of Zinc, known to promote healthy hair and nail growth (Khan *et al*., 2011). Another befitting hypothesis could be the presence of certain proteins and amino acids in the leaves of *T. foenum-graecum* that may promote hair growth (Purwal *et al*., 2008). It is also plausible that Trigonelline, an alkaloid present in the leaves of *T. foenum-graecum,* which physiologically interacts and increases blood circulation to the scalp, maypromote hair growth in mice with alopecia (Gupta *et al*., 2013). Additionally, estrogen has been shown to modulate several transcription factors known to control hair growth (Ohnemus *et al*., 2006), thus, it is highly likely that steroidal saponins like diosgenin having estrogen like effects in *T. foenum-graecum* leaves might contribute towards hair growth promoting effects in our mice (Sharma

et al., 2009). Possibly, these phytoestrogen interacts with dihydrotestosteron (DHT) metabolism and increases hair growth (Schulz *et al*., 2006). Besides, it has also been proposed that rather than synthetic hormone, which causes side effects, phytoestrogens can be used as an alternative to promote hair growth with minimal side effects (Dixon, 2004; Ross *et al*., 2000).

CONCLUSION

Thus, in conclusion, our data suggested that the extracts of leaves of *T. foenum-graecum* significantly improved hair growth in mice in comparison to standard and most frequently chosen synthetic hair tonic *i.e.,* minoxidil, in ethanol (5% and 10%) and petroleum ether (5%) extracts. Presumably, the effects of leaves extracts are superior in comparison to extracts made from *T. foenum-graecum* seeds. However, it would be nice to perform a side by side comparison between leaves and seeds extracts of *T. foenum-graecum*. Besides, further studies are required to un-earth the underlying mechanisms of hair growth promoting effect of *T. foenum-graecum* leaves, particularly, by identifying constituents of the extracts responsible for hair growth promoting effects.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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