Original Article

Preparation and evaluation of a hair wax containing propolis and *Eruca sativa* seed oil for hair growth

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Abstract

Background: Hair growth as a key consumer objective has important role in the hair care products researches. This study was aimed to investigate the effect of a hair wax containing propolis, a resinous mixture produced by honeybees in *Eruca sativa* seed oil base on hair growth.

Materials and Methods: The hair wax was designed and formulated compared with marketed brand hair wax and evaluated for pharmaceutical parameters including pH, homogeneity, consistency, spread ability, *in vitro* drug release, and stability. After selection of the best formulation containing 10% ethanolic extract of propolis and 10% *E. sativa* seed oil, the hair growth potential was evaluated by application of 1 g hair wax daily on 4 cm^2 area of dorsal side of Wistar rats and compared with controls and standard medication (1 ml of 2% minoxidil). After 30 days treatment, the length and weight of hairs and percentage of hair follicles in different phases of growth in skin biopsies were assessed.

Results: The selected hair wax formulation was stable and easy to wash. The formulation significantly increased hair length on 10^{th}, 20^{th}, and 30^{th} day compared control group (5.8 ± 0.3 vs. 2.6 ± 0.4, 11.4 ± 0.6 vs. 5.8 ± 0.4, and 17.5 ± 0.5 vs. 12.7 ± 0.4 mm, respectively) and also the weight of newly grown hairs on 30^{th} day (0.056 ± 0.006 vs. 0.043 ± 0.005). It improved hair follicles percentages in anagen phase without any sensitivity reaction.

Conclusions: The results of this study suggest that the formulated hair wax containing of propolis and *E. sativa* seed oil could have significant effect on promoting hair growth.

Key Words: *Eruca sativa* Mill., hair growth, hair wax, propolis

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INTRODUCTION

Hair is an external appendage of skin that derived from ectoderm and grows from hair follicles containing of root, shaft, and tip.[1] Hair growth occurs during three different stages including anagen (long growing phase), catagen (brief transitional apoptotic phase), and telogen (resting phase) phases.[2]

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Alopecia or hair loss may occur when a person lose more than 100 hairs/day. Alopecia can be classified as focal or diffuse and comprising a large group of disorders with various etiologies. In spite of various medical treatments and many hair care products, many people suffer from this dermatologic disorder worldwide. Therefore, it is of great concern to discover novel products for stimulation of hair growth and preventing hair loss.[3,4]

Propolis is a natural compound produced by honeybees containing over 200 chemical compounds including flavonoids, terpenoids, aldehydes, aromatic acids, aliphatic alcohols and ethers, amino acids, and sugars.[5] Different pharmacological activities including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, hepatoprotective, immunostimulating, and antitumor properties have been reported for propolis.[6,7] Regarding the presence of various bioactive components, propolis has many helpful effects on human health. It also has the potential for use in the treatment of dermatological disorders and is found in hair and skin products for repairing and regenerating of damaged tissues.[8,9]

Eruca sativa Mill. belongs to the *Brassicaceae* family is one of the most important oil seed crop with a large variety of medicinal and therapeutic activities.[10] Various phytochemical constituents including steroids, terpenoids, coumarins, flavonoids, and isothiocyanates have been identified in *E. sativa* seed oil.[11,12] There are also some important fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid, and erucic acid in seed oil of this plant.[10] In Iranian traditional medicine, *E. sativa* is called Mandab and used as aphrodisiac, anti-inflammatory, antifungal, and anti-infectious for dermal problems and for treatment of ulcers, scurvy, mange, hair loss, and hair lice.[10,13-16]

The present study was designed to evaluate the hair growth potential of an oil-in-water (O/W) emulsion-based hair wax formulation containing propolis and *E. sativa* seed oil.

MATERIALS AND METHODS

Materials

Propolis was purchased locally from the markets in Yasooj, Kohgiluyeh, and Buyer Ahmad Province, Iran. Mandab oil was purchased from a local market in Yazd, Iran. Beeswax was obtained from Abyaneh Cosmetic Company (Isfahan, Iran). Folin–Ciocalteu reagent and all other chemicals were obtained from Sigma-Aldrich Chemical Co., (Steinheim, Germany) or Merck Co., (Darmstadt, Germany).

Wax extraction and preparation of ethanolic extract of propolis

Propolis (20 g) was melt in isopropyl myristate (200 mL) at 80°C. The mixture was filtered and kept in room temperature for 24 h. After removal of solvent with refiltration, the obtained wax was stored at 4°C for 48 h to increase its consistency.

Maceration method was used for the preparation of ethanolic extract of propolis in the proportion of 30 g of propolis to 300 mL of solvent (ethanol 96%) for 6 days at room temperature. Then, suspension was filtered and stored in 4°C. After 24 h, extract was filtered and condensed by rotary evaporator.[17] The obtained extract was freeze-dried and the residue was weighed.

Estimation of total phenolic content

The Folin–Ciocalteu method was used for the determination of total phenolic content of propolis extract. Briefly, the freeze-dried propolis extract was dissolved in ethanol and diluted with water. Then, the sample was mixed with sodium bicarbonate (20%) and diluted Folin's reagent. After incubation for 2 h at room temperature, the absorbance was measured at 765 nm against the reference blank. Total phenolic content was estimated from calibration curve obtained from different concentrations of gallic acid and expressed as gallic acid equivalents (GAE) per gram of dried extract.[18]

Characterization of *Eruca sativa* seed oil

E. sativa seed oil properties including acid value, iodine value, saponification value, peroxide value, and refractive index were determined according to the standard methods.[19-21] Refractometer (Bellingham, Stanley, England) was used for the determination of refractive index.

Formulation of the hair wax

An O/W emulsion-based hair wax containing ethanolic extract of propolis and *E. sativa* seed oil was formulated. For the development of a suitable emulsion, several formulations were prepared using different concentrations of ticking agents of the oil and aqueous phases [Table 1]. The extracted wax from propolis was used to increase the consistency of formulations. The oil and aqueous phases were separately prepared and heated up to 75°C. Then, aqueous part was added to the oily part while stirred continuously. Formulation was stored at room temperature to cool and essence of cocoa was slowly added to cover odor of *E. sativa* seed oil.[22,23] Concentrations of thickener agents were increased to achieve suitable consistency. The consistency of the formulations was determined by a penetrometer (Stanhope–Seta, Surrey, England) at room temperature for 5 s and compared with a

Table 1: Hair wax formulations

Ingredients	F1	F2	F3	F4	F5	F6
Ethanolic extract of propolis (ml)	10	10	10	10	10	10
E. sativa seed oil (ml)	10	10	10	10	10	10
Extracted waxes from propolis (ml)	3	3	4	4	5	5
Yellow bees wax (mg)	2	3	4	4	4	5
Cetyl alcohol (mg)	3	3	4	5	6	7
Glycerin (ml)	3	3	3	4	4	4
Tween 80 (ml)	2	2	2	2	2	2
Cetrimonium chloride (ml)	3	3	3	3	3	3
PVP (mg)	3	3	3	4	4	4
PEG 6000 (mg)	30	31	32	33	34	35
Essence of cocoa (ml)	1	1	1	1	1	1
Purified water up to (ml)	100	100	100	100	100	100

marketed hair wax (bone hair wax). In this way, under defined time, the penetration is measured as the depths in millimeters to which a standard penetrant such as a cone or a needle immerges into a semi-solid material. The best formulation was selected and then analyzed by other tests.

Evaluation of selected hair wax formulation

The physical appearance of hair wax formulation was visually inspected for color and homogeneity. The pH value of prepared formulation was measured by a digital pH meter (Metrohm, Switzerland). Consistency of the formulation was determined as defined previously. The spreadability was measured as spreading-diameter using parallel-plate method.[23] For this mean, two glass plates (41/8 g and 43/03 g) were used. One gram of prepared formulation was placed on one of the plate and its diameter was measured. Then, other plate was placed on it. New diameter was measured after 1 min.

Total phenolic content of selected formulation was estimated using Folin–Ciocalteu method as a measure of drug content.

In vitro drug release study was carried out in Franz diffusion cell containing 25 ml of phosphate buffer (pH 7.4) as receptor medium which was kept at 37 ± 1°C and stirred by magnetic stirrer.[24] The selected formulation (0.5 g) was uniformly spread on the cellulose acetate membrane. Intervals of 0.5, 1, 2, 3, 4, 5, and 6 h were used for sampling. In each time, 1 mL of receptor medium as sample was removed and replaced with an equal volume of fresh receptor medium immediately. Then, Folin–Ciocalteu method was used to measure the total phenolic content of the samples. To define drug release kinetics of selected formulation, zero-order, first-order, and Higuchi kinetic models were used to analyze the results of drug release.[21,22] For this mean, the regression coefficients were calculated for different kinetic models and the best one was considered as the fitted model for the drug release kinetic of the selected formulation.

To investigate the effects of temperature, humidity, and time on selected formulation, it was stored at 40°C and 75% relative humidity (RH). After 1 month, appearance, pH, consistency, and drug content of formulation was determined at room temperature for assessing the stability.[22]

Animals

Male Wistar albino rats, weighing 200–250 g obtained from the animal house of the School of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran) were used for hair growth study. The animals were housed under standard laboratory conditions. All animal experiments were approved by the Institutional Animal Ethical Committee of Isfahan University of Medical Sciences.

Primary skin irritation test

Hairs from 4 cm^2 area of dorsal side of the rats were shaved, and surgical spirit was used to complete removal of the hairs. About 1 g of selected formulation was applied over the location. After 48 h, the test site was observed for erythema and edema.[25]

Hair growth evaluation

The rats were divided into five groups of five rats each. Hairs from 4 cm^2 area of dorsal side of the rats were shaved and surgical spirit was used to complete removal of hair. Group 1 used as control (without medication), Group 2 was treated with standard medication (1 ml of 2% minoxidil ethanolic solution daily), Group 3 was treated with formulated hair wax containing *E. sativa* seed oil and propolis (1 g daily), Group 4 was treated with hair wax containing *E. sativa* seed oil (1 g daily), and in Group 5, 1 g of hair wax base containing liquid paraffin (common base in marketed hair waxes) was applied over the shaved area of dorsal side of rats for 30 days. Regrowth of the hairs was visually detected at 10^{th}, 20^{th}, and 30^{th} day after topical application of hair formulations.[25]

Hair length determination

On 10, 20, and 30 days after beginning the treatment, hairs were randomly plucked from the shaved area of animals from all groups and the average length of 10 hairs was measured.[25]

Hair weight determination

After 30 days, the rats were sacrificed and 1 cm^2 area of selected dorsal site was cut and weighed. Then, the hairs of the cut area were shaved and re-weighed. Hair weight was determined by calculating the difference between skin weight without hair and skin weight with hair.[25]

Histological study

After 30 days, skin biopsies were obtained from the shaved area of the rats and fixed in 10% formalin. The specimens were embedded in paraffin and sectioned into thickness of 10 μm. After staining of slices with hematoxylin and eosin, the number of hair follicles per millimeter area of the skin and ratio of hair follicles in different phases of growth including anagen (active growth phase) and telogen (resting phase) was determined microscopically.[26]

Statistical analysis

Results were reported as the mean ± standard error of mean. For data analysis, one-way analysis of variance followed by Tukey *post hoc* test was performed using SPSS software Version 16.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

The yield of the wax extraction from propolis by isopropyl myristate was 32% (w/w). The yield of dried ethanolic extract of propolis obtained after freeze drying was 38% (w/w). Total phenolic content of ethanolic extract of propolis was 136.57 mg GAE per gram of dried extract.

Physiochemical properties of *E. sativa* seed oil were shown in Table 2. Six formulations were prepared and appropriate consistency by increasing the amount of thickener agent and comparing with marketed hair wax were selected. The formulation F_6 containing of 10% *E. sativa* seed oil and 10% ethanolic extract of propolis was selected as the best one and used for hair growth evaluation.

The selected formulation (F_6) was yellowish semi-solid, homogeneous, and without palpable particles. Its pH was in the range of human hair and scalp oil (pH = 4.5–5.5). Table 3 shows the result of quality control examinations of the selected formulation. Formulation F_6 had the most similar consistency and spreadability as compared with marketed hair wax (32 vs. 30 and 35 ± 3 vs. 32 ± 2, respectively).

Drug release study for selected formulation was carried out in Franz diffusion cell, and after 6 h, 86.49% of polyphenols were released [Figure 1]. The results of the correlation coefficient kinetic and rate constant in different models are shown in Table 4. The most appropriate kinetic was Higuchi kinetic model. According to Table 5, the selected formulation F_6 was stable after maintenance at 40°C and 75 RH for 1 month and the results were satisfactory.

Table 2: Physiochemical properties of *Eruca sativa* seed oil

Tests	Results
Acid value (mg KOH/g)	0.79
Iodine value (g/100g)	107.2
Saponification value (mg KOH/g)	178.4
Peroxide value (mEq/Kg)	8.3
Refractive Index	1.491

Table 3: Physicochemical properties of the selected formulation (F6)

Parameters	Results
Physical appearance	Yellowish semisolid, homogeneous
pH	5.1±0.2
Drug content (mg GAE/g dry extract)	11.51
Consistency (penetration of penetrometer/mm)	32
Spreadability (difference between the initial and final diameter/mm)	35±3

Table 4: Kinetic parameters of selected formulation release

Zero-order release		First-order release		Higuchi equation	
K_0	R^2	K_1	R^2	K_h	R^2
13.427	0.9294	0.0967	0.9052	36.504	0.9971

Table 5: Stability study parameters of selected formulation

Parameters	Initial	After one month 40/70(°C/RH)
Appearance	Yellowish semisolid, homogeneous	Yellowish semisolid, homogeneous
pH	5.1	5.2
Consistency (penetration of penetrometer/mm)	32	32
Drug content (mg GAE/g dry extract)	11.51	11.49

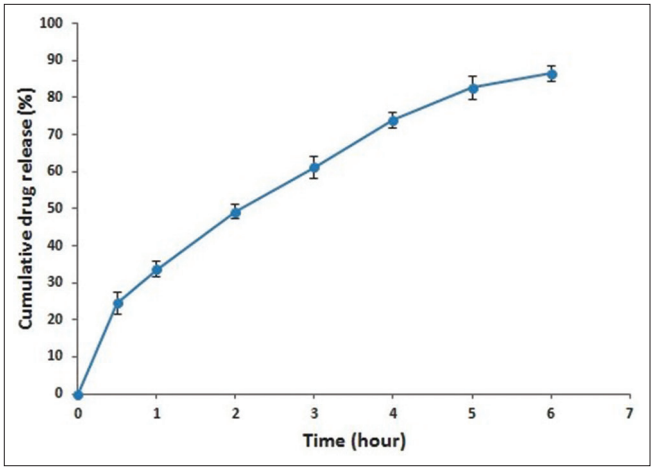

Figure 1: Percentage of cumulative drug release of selected formulation (F6) in Franz diffusion cell through a cellulose acetate membrane at 37°C during 6 h. Values are expressed as mean ± standard error of mean

In animal study, results from primary skin irritation test did not show any sensitivity reaction in and the selected formulation was considered safe for topical application.

The length of hairs was measured in five groups of animals on 10th, 20th, and 30th day of treatment [Figure 2]. There was the lowest hair length in Group 1 (control group with no treatment) (12.7 mm). Treatment with selected formulation containing propolis and *E. sativa* seed oil also showed significant effects on hair length on 10th and 20th day ($P < 0.01$ and $P < 0.001$, respectively).

The weight of newly grown hairs was determined in all animals on 30th day of treatment [Figure 3]. The weight of hairs for Group 2 (minoxidil 2%), Group 4 (selected formulation F_6), and Group 3 (*E. sativa* seed oil) was significantly more than liquid paraffin-treated group and control group.

The effect of all treatments on hair growth was evaluated visually [Figure 4] and also histologically by estimating different phases of hair growth [Figures 5 and 6]. After 30-day treatment, the percentage of hair follicles in the anagen (active growth) phase in minoxidil-, F_6- and *E. sativa* seed oil-treated groups was significantly more than liquid paraffin-treated group and control group.

DISCUSSION

Hair plays an important role in the beauty and personal charm, so today, the production of hair care products and cosmetics is increasingly considered.[27] Development of new hair growth promoters is one of

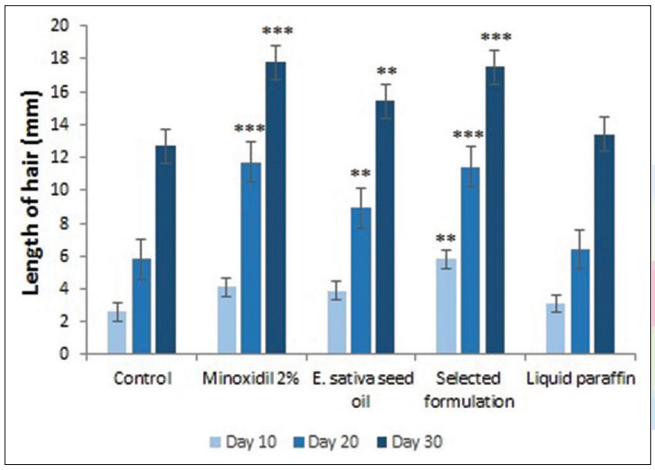

Figure 2: Effects of various treatments on hair length of Wistar rat at different times. Values are expressed as mean ± standard error of mean $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control group

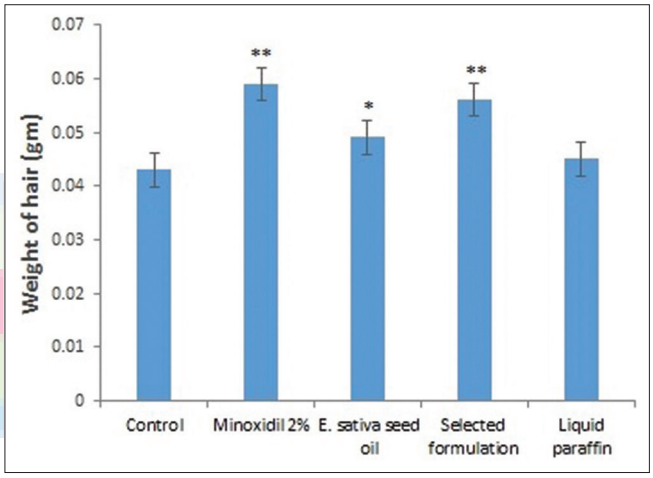

Figure 3: Effects of various treatments on hair weight of Wistar rat. Values are expressed as mean ± standard error of mean $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group

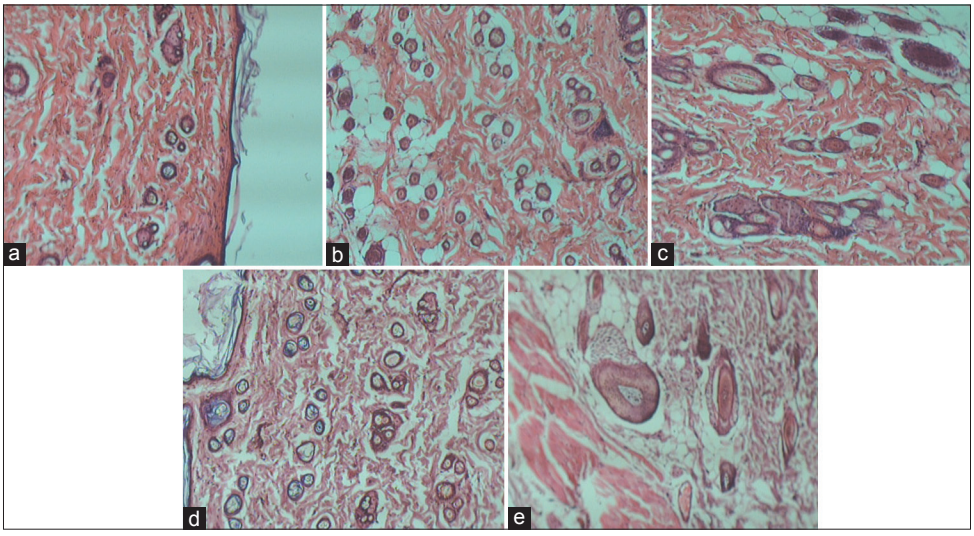

Figure 4: Skin histology sections of Wistar rats; (a) control, (b) minoxidil 2%, (c) hair wax base containing *Eruca sativa* seed oil, (d) selected formulation containing propolis and *Eruca sativa* seed oil, (e) hair wax base containing liquid paraffin

the greatest needs since there are only two medications including minoxidil (topical) and finasteride (oral) have been approved by the FDA for the treatment of hair loss.[28] A randomized control trial conducted by the Upjohn Company demonstrated that minoxidil, a potassium channel opener, has been effective on 54% of patients. Significant adverse reactions include pruritus, dryness, scaling, local irritation, and dermatitis have been reported in patients treated with topical minoxidil.[29] In addition, long-term treatment with minoxidil is needed and the most effect has been seen after 1 year with declining in subsequent years.[30] For the treatment of androgenic alopecia in men, finasteride, an inhibitor of 5-alpha reductase, has been effective for hair growth in 48% of patients in a 1-year clinical trial study reported by Merck research laboratories. Finasteride is well tolerated but some patients have discontinued it due to sexual disorders.[4,31] According to report of the American Hair Loss Association, loss of hair leads to emotional problems and vulnerability of the patients.[32] These issues have been led to the efforts for developing more effective anti-hair loss agents with fewer side effects for the treatment of hair loss.

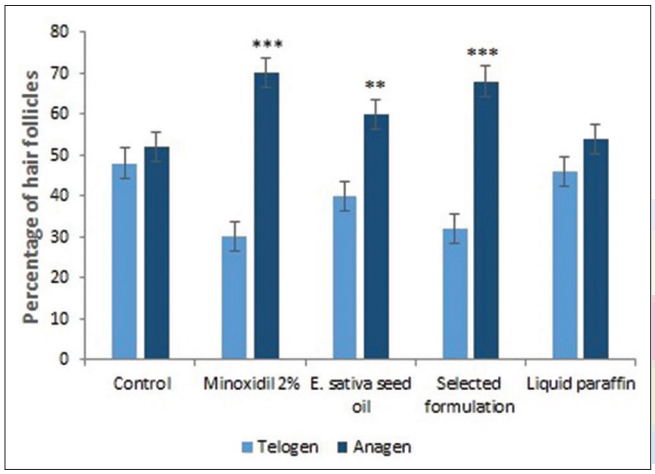

Figure 5: Effects of various treatments on the percentage of hair follicles in the anagen and telogen phases. Values are expressed as mean ± standard error of mean *$P < 0.01$ and **$P < 0.001$ versus control group

In the present study, the formulated hair wax for hair growth containing propolis and *E. sativa* seed oil was stable and easy to wash. Results from *in vivo* study showed the effects of *E. sativa* oil in hair wax base to increase the weight and length of hairs and hair follicles percentages in anagen phase. This effect may be due to the premature shifting of hair follicles from the telogen to anagen phase.[33] Bioactive constituents of this plant seed oil may be involved in its hair growth activity observed in this study. Shimizu *et al*. have shown that fatty acids such as linolenic acid and stearic acid had 5α-reductase inhibitory activity and hair regrowth stimulatory effects.[34] Hair wax containing propolis and *E. sativa* oil possessed greater potential in promoting hair growth that could be due to the high amounts of polyphenols. Our results showed the total phenolic content of ethanolic extract of propolis as 136.57 mg GAE per gram of dried extract. Ramanauskiene *et al*. also reported the similar result for total phenolic content of propolis extract as 115.4 mg GAE per gram of dried extract.[35] A number of researchers have found that flavonoids stimulated hair growth by increasing blood flow and nourishing the hair follicles.[25] Animal studies have shown the effect of topical application of propolis on hair regrowing and increasing the number of special cells involved in the process of growing hair in mice. Abnormal inflammation has a role in hair

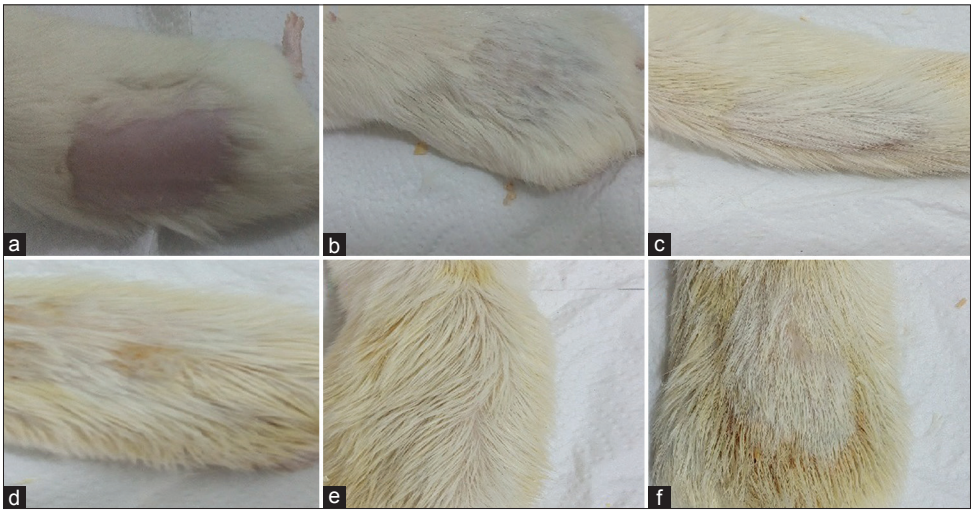

Figure 6: Effects of various treatments on hair growth of Wistar rats on the 1st day (a) and after 30 days; (b) control, (c) minoxidil 2%, (d) hair wax base containing *Eruca sativa* seed oil, (e) Selected formulation containing propolis and *Eruca sativa* seed oil, (f) hair wax base containing liquid paraffin

loss conditions and anti-inflammatory activity of propolis may be contributed to its helpful effects on hair growth. Topical propolis has also resolved alopecia caused by dermatophytes. Antifungal effect of propolis has been proposed as another possible mechanism for its hair growth potential activity.[36] It has been suggested that not only local application but also oral treatment by different honeybee products including honey, propolis, pollen, and royal jelly may be useful to treat alopecia.[37]

CONCLUSION

Results of this study showed that the hair wax formulation containing propolis and *E. sativa* oil was pharmaceutically stable and well compatible with skin without causing sensitivity reaction. It significantly improved hair growth in animal model and may have promoting effect on hair growth in humans.

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Conflicts of interest
There are no conflicts of interest.

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