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RESEARCH ARTICLE

EFFECTIVENESS TEST OF FENUGREEK SEED (*Trigonella foenum-graecum* L.) EXTRACT HAIR TONIC IN HAIR GROWTH ACTIVITY

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ABSTRACT

There are many causes of hair loss, among others is estrogen deficiency. External estrogen administration could change the hormonal cycle and increased cancer risk. One of the natural alternative estrogen therapy can be found in various plants containing natural product among which are compounds with weak estrogenic activity, termed phytoestrogen. Phytoestrogen compete with estrogen by filling or binding to the estrogen receptor and producing the estrogen effect. Phytoestrogen in fenugreek seeds (Trigonella *foenum-graecum* L.) is believed to increase hair growing process; however, up to now there is no scientific study to prove it. Therefore, the objection of this study is to prove the effect of hair tonic containing fenugreek seeds extract in different concentration on hair growing activity of New Zealand strain rabbit; and to get the optimal concentration of fenugreek extract as well as the safety data. Hair growing activity is determined by hair length, hair diameter and hair weight measurement, while toxicity test is determined by Draize skin test and Draize eye test. The result of the activity test using 10% fenugreek extract seed hair tonic showed significant difference ($p < 0,05$) compare to placebo and resemble the result using minoxidil 2% hair tonic. Sensitivity test results showed mild irritation effect.

INTRODUCTION

The hair has a protective role against adverse effects of the environment, for example towards the temperature and ultraviolet (UV) light (Harahap, 2000). The most significant role of the hair is for aesthetic purpose and thus, if the hair encounters any abnormalities, the confidence level of that person will be disturbed. The most common abnormality include depigmentation (gray-hair), dandruff, and hair loss. Hair loss is the reduction of hair volume which causes hair thinning and even baldness. Hair treatment using merely shampoo and conditioner is not enough as hair roots are living cells that need to be nourished in order to stay healthy; therefore, the administration of hair tonic is also required (Wasitaatmadja, 1997). Hair tonic is a cosmetic preparation to reduce hair loss, to stimulate, and to increase the hair volume. The main components in tonic preparation are the solvent and active ingredients. The most common solvent used for liquid preparation are water, alcohol, and butylene glycol. The effects of active ingredients used in hair tonic are to clean, to prevent or to eradicate dandruff, to repair the blood circulation in the scalp and stimulate hair growth (DKRI and DJPOM). Indonesian people tend to use herbal products since the side effects are less as compared to those of synthetic products, such as minoxidil, which often cause hypersensitivity of the scalp (Purwal *et al.*, 2007). Indonesia is a country which is abundant of plants and vegetation that may serve as the ingredients to produce hair tonic. Klabet (*Trigonella foenum-graecum* L) is one of the plants in Indonesia, also known as *fenugreek*, which was first found in the Mediterranean area and commonly cultivated in northern Africa and India. The seed of fenugreek is yellow and is bitter in taste (Heyne, 1987). Fresh fenugreek leaves are useful in treating digestive problems and bloating stomach. The extract of fenugreek leaves, if applied regularly to the scalp before shower, is also shown to enhance hair growth, preserve the hair's natural color, soften the hair, and treat dandruff (Yadav and Kaushik 2011). In the hair growth process, the estrogen hormone, besides steroid and androgen, has a significant role (Ohnemus *et al.*, 2006); however, the use of synthetic hormone may cause many side effects (Ross *et al.*, 2000). The fenugreek seed contain sapogenic steroid; disogenin, glitogenin, and tipogenin, which have estrogenic/sex hormone precursor effects (Evans, 2002). Plant-derived-estrogen/phytoestrogen is a rational alternative to reduce the side effects (Dixon, Richard 2004). Phytoestrogen from fenugreek plant is thought to diminish hair loss and increase hair growth rate. The objectives of this study are to prove whether fenugreek seed have any effect on hair growth, to obtain the optimum concentration of fenugreek seed extract in hair growth process, and to gather the sensitivity data (*Draize skin test* and *Draize eye test*).

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Study Method

Simplisia *Trigonella foenum-graecum* (klabet) was obtained from Herbal Research Center (*Balai Penelitian Tanaman Obat (BPTO))*, Tawangmangu, Central Java.

Chemical Ingredients

Minoxidil, Ethanol 98%, distilled water, butylene glycol, Microcare PM5®(INCI name: phenoxyetanol, methylparaben, ethylparaben, propylparaben, isobutylparaben andbutyl paraben), Solubilant LRI® (INCI name: PPG-26 Buteth-26 and PEG-40 *hidrogenated castor oil* & *water*), HCl 2 N, Bauchardat reagent, Mayer reagent, Dragendorff reagent, methanol 50%, magnesium (Mg), concentrated HCl, $CHCl_3$, H_2SO_4, anyhdrate acetic acid, $FeCl_3$ solution, Lead (II) acetate 0,4 M, isopropanol, anyhydrate sodium sulphate, Molish LP.

Equipment

Analytic weighing scale (Adam AFA-210 LC), macerator, *soxhlet (Buchi R-3), rotary evaporator buchi R-250*, parchment paper, beaker glass, measuring glass, spatula, evaporating basin, watch glasses, digital vernier caliper (*Mitutoyo digimatic caliper*), *sendok tanduk*, homogenizer, pH meter (Eutech), microscope with micrometer Nikon Eclips E 200, SEM (*Scanning Electron Microscope*).

Making of The Extract

This study uses hot soxhlet extract, which is an extraction process with a relatively constant solvent along with a condenser. First, fenugreek seeds were grinded using a mixer and using a weighing scale, 100 grams were taken and put into a parchment paper and then extracted with 350 ml 98% ethanol using a soxhlet. The extraction process was done until the solution color in the circulator became colorless. The sample extract was then concentrated using rotary evaporator buchi R-250 and weighed.

Fenugreek Seed Extract Photochemistry Identification

Extract phytochemistry identification is a form of analysis to detect the presence of chemical compound in a particular extract. The compound which were investigated in this study were alkaloid, saponin, tannin, triterpenoid, and glycoside.

Alkaloid

The alkaloid examination was done by dropping 8 drops of extract into a reaction tube with 1 ml HCl 2 N and 9 ml aquades inside. Then, the tube was heated on a 60^{o}C water bath for 15 minutes. The solution was then cooled and filtered and was called solution A. 3 drops of solution A were put into a reaction tube and 1 drop of Bauchardat was applied. If a brown-black precipation was formed, then alkaloid was said to be present. 2 drops of Mayer reactant was also added into 3 drops of solution A and if alkaloid was present, a white or yellow precipitation in the form of clotting would occur. 2 drops of Dragendorff reactant were also added into 3 drops of solution A and would form red or pink precipitation. If at least 2 out of the 3 tests were positive, then the solution could be said to contain alkaloid (Septiyaningsih, 2010).

Saponin

2 ml of the fenugreek seed extract was diluted with distilled water with a 1:1 ratio and shaken for 10 minutes. If the foam persisted for 30 minutes, then saponin was present in the extract.

Flavonoid

3 ml of the fenugreek seed extract was diluted in 1-2 ml 50% methanol and both magnesium and 4 drops of concentrated HCl were applied into it. If the solution turns red or pink, then flavonoid could be said to present in the solution (Septiyaningsih, 2010).

Triterpenoid

3 ml of the fenugreek seed extract was added with 0.5 ml chloroform ($CHCl_3$) and 1-2 ml sulphuric acid (H_2SO_4). 0.5 ml anhydrate acetic acid was added into this solution along the wall of the reaction tube. The presence of trterpenoid was indicated by the presence of brownish ring or violet between the 2 solvents (Hayati, 2008).

Tanin

3 ml of the fenugreek seed extract was diluted with 2 ml distilled water and 3 drops of $FeCl_3$ solution were added. The occurence of black blue color indicated the presence of gallotannin compound while black green color indicated the presence of catechin tannin (Septiyaningsih, 2010).

Glycoside

Three grams of fenugreek seed extract was mixed with 30 ml solution of 7 parts ethanol (95%) and 3 parts water inside a water cooler for 10 minutes and then cooled down and filtered. Afterwards, 25 ml distilled water and 25 ml Lead (II) acetate 0.4 M were added to 20 ml filtrate, shaken, and left for 5 minutes before filtered. The filtrate was filtered 3 times, each time with 20 ml mixture of chloroform P and isopropanol P of 3:2 ratio. Then, anhydrate sodium sulphate was added to the filtrate, filtered, and evaporated in a temperature of not more than 50^{o}C and the remaining was diluted with 2 ml methanol. This solution was then called solution A. To examine the presence of glycoside, about 0.1 ml solution A was evaporated and the remaining was diluted in 5 ml anhydrate acetic acid and 10 drops of sulphuric acid were added. If the solution turned blue or green, it showed a positive result. To examine the presence of carbohydrate, about 0.1 ml solution A was evaporated and 2 ml of distilled water, 5 drops of Molish LP, and 2 ml of sulphuric acid were added into the remaining. If a purple ring was formed, the test (molish reaction) was said to be positive (Badan Pengawas Obat dan Makanan, 2006).

Synthesis of The Hair Tonic

The composition of the hair tonic is listed in Table 1. The fenugreek seed extract was diluted in distilled water. The preservatives was diluted in ethanol and then was added into the mixture of distilled water and fenugreek seed extract, stirred, mixed with solubilizer, and then was stirred again. Afterwards, a little distilled water was added again and the

solution was stirred until it became homogenous. Butylene glycol was then added into it. Finally, distilled water was added into this mixture and then stirred until it became homogenous.

The Evaluation of The Hair Tonic

a.Organoleptic Test
Identification using the senses and covered the smell and color of the hair tonic.
b.Homogeneity Test
The hair tonic was smeared on to a clean and dry object glass and covered with a cover glass. The presence of any coarse particle/in homogeneity was examined under light.
c.pH Test
A pH meter was calibrated using a buffer solution with pH 4 and pH 7. The electrode was immersed into the hair tonic and left for a few minutes until the pH stabilized

Preparation of The Experimental Animal

This study used male rabbit since the esterogenic effect was less than that of female. New Zealand breed rabbits with the weight of approximately 2,000-2,500 grams were obtained from *Balai Penelitian Ternak*, the Center of Animal Study, Ciawi, West Java. Before any examination was done, the rabbits were first acclimated for 2 weeks. Every rabbit was numbered using the *Permanent Marker Artline*® marker on the head region. The rabbits were kept in plastic cages with wood shavings as their bases to absorb the feces. They were fed everyday with pellets and distilled water as their drink as much as they wanted (*ad libitum*). The plastic cages with the rabbits inside were put in the animal house of the Histology Faculty of University of Indonesia. The cages were cleaned three times a week by washing it with soap, soaked into disinfectant solution, and dried. The base of the cage was also changed with new wood shavings. There were timers in the cages to regulate the lighting, dark or bright, each for 12 hours.

Skin Sensitivity Examination (Draize Skin Test)

This skin sensitivity examination was an initial examination to detect any allergic reaction that may be caused by the hair tonic. Hair on the right and left back were shaved and divided into 6 regions, each with a size of 4x4 cm in a rectangular shape with a distance of 1.5 cm between the squares. Each square was numbered 1-6. After shaving and before any hair tonic was applied, ethanol was applied to the skin as an antiseptic. Number 1-6 represent:

1.Number 1 → no tonic was applied/normal control
2.Number 2 → negative control was applied/placebo
3.Number 3 → Fenugreek seed extract hair tonic 2.5% was applied
4.Number 4 → Fenugreek seed extract hair tonic 5% was applied
5.Number 5 → Fenugreek seed extract hair tonic 10% was applied
6.Number 6 → Minoxidil 2% was applied (positive control)
Any sensitivity of the skin, such as erythema, edema, and peeling was observed at 24 hours and 48 hours after the application.

Test the sensitivity of the eye *(Draize eye test)*

3 drops of fenugreek seed extract 2.5% in physiologic NaCl were given into the rabbit's left eye (as the control is the right eye). Observations were made in 30 minutes, 60 minutes, 120 minutes, 240 minutes, 1 day, 2 days, 3 days and 4 days. The cornea, iris and conjunctiva scores were calculated (Eaton, 2001).

Hair growth activity test

This test was done on 3 areas on each side, right and left, of the rabbits' shaved back with a size of 4x4 cm each. Then, depilatory cream (Veet® cream) was applied for 3-5 minutes and that area was rinsed with water until that area is clean with hair. 70% ethanol was then applied as an antiseptic. The rabbit was left for 24 hours before any activity test was done. Treatment 1 was the normal control as no intervention was done. Treatment 2 acted as the negative control, where hair tonic containing no test substance was applied and fenugreek seeds extract 2.5%, 5%, and 10% was applied to treatment 3, 4, and 5, respectively. Last, hair tonic containing minoxidil 2% as the positive control was applied as the positive control. 0.1 ml of each treatment was then applied to the rabbit twice a day for 3 weeks. The first day of the hair tonic application was considered as day 0.

a. Qualitative assessment of hair growth

Qualitative assessment of hair growth analysis was performed by visual observation of 2 parameters, the initial time of hair growth (minimum time required for the growth of hair on the shaved area, assessed from the darkening of the skin color, which showed initial hair growth) and hair growth completion time (minimum time required for the entire shaved area to be covered with new hair).

b. Observations of the growth of hair length

Done by taking 10 random hair strands on each box on the day-7, 14 and 21. The hair was pulled, straightened out, and attached to a tape. Measured using digital calipers *Mitutoyo Digimatic* brand caliper brands. The average length obtained was analyzed to see whether there was a statistically significant difference between the test area with the control (Adhirajan *et al.*, 2003).

c. Hair diameter measurements

Hair diameter was measured with a micrometer microscope Nikon Eclipse E200. The average hair diameter was processed statistically (Adhirajan *et al.*, 2003).

d. Hair weight measurements

Hair were removed and weighed on day 21 to determine the weight from each box and then calculated statistically (Adhirajan *et al.*, 2003).

Solubilizer Test

The hair tonic formula used in this test same test was the same as that of Table 1, without the addition of fenugreek extract.

Table 1. Hair Tonic Formulation

Ingredient	Consentration (%) (b/b)				
	Control - / placebo (%)	Formula A (%)	Formula B (%)	Formula C (%)	Control + (%)
Klabet extract	-	2,50	5,00	10,00	-
Minoxidil	-	-	-	-	2,00
Buthylene glikol	2,50	2,50	2,50	2,50	2,50
Preservative	0,50	0,50	0,50	0,50	0,50
Solubilizer	3,10	3,10	6,20	11,20	-
Ethanol 96%	25,00	25,00	25,00	25,00	25,00
Aquadest	69,50	66,40	60,80	50,80	70,00

Table 2. Draize eye test Assessment

Eye Lesion Gradation	Score
I. Cornea Score	
OL: Opacity level	
No opacity	0
Spotted or generalized opacity	1
The details of the iris are clearly seen	
Translucent area looks clear, the detail of the iris is vague	2
Translucent areaisgreyish white, iris details are not clear or the iris is clear, pupil size is difficult to see	3
Opaque cornea, iris is not visible	4
Opacity area: OA	
¼ < x < 0 bagian	1
¼ < x < ½ bagian	2
½ < x < ¾ bagian	3
¾ < x < 1 bagian	4
II.Iris score	
I:Iris score	
Normal	0
Folds above normal, congestion, swelling, circum corneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
Not much reaction towards the light, bleeding occurs, major damage (one of them or any combination)	2
III. Conjunctive score	
R : Reddening (only based on conjunctiva palpebra)	
Normal blood vessel	0
Blood vessel exceeds normal	1
Larger, chrimson red color, single blood vessel is not easily seen	2
Dark red, diffuse	3
C : Chemosis (cover eye lid and/nictitating membrane)	
No swelling	0
Swelling exceeding normal (including the nictitating membrane)	1
Clear swelling with the eye lid partly lifted	2
Swelling with the eye lid partly closed	3
Swelling with the eye lid more than half-closed	4
L: Lacrimation (tears production)	
No tears	0
Some tears present, distinguishable from normal condition	1
Lacrimation that moistens the eye lid and eye hair	2
Lacrimation that moistens the eye lid and areas around the eye	3

1.Formula A: normal control
2.Formula B: addition of 3.1% solubilizer.
3.Formula C: addition of 6.2% solubilizer.
4.Formula D: addition of 11.2%solubilizer.

Shaved back of rabbits was divided into 4 regions, each of a rectangular shape with 4 cm x 4 cm size and 1.5 cm spacing between boxes. After shaving and before bathing, the rabbits' backs that had been divided were smeared with ethanol as an antiseptic. Parts of the area are:

Region I : not oiled / normal control
Region II : formula A was applied
Region III : formula B was applied
Regions IV: formula C was applied

0.1 ml of each formula was given twice a day for 3 weeks. The first day was considered as day 0. Observations were made by taking a random hair on each box on day 7, 14 and 21. The hair was pulled out, straightened, and stuck on tape. Measured was done with digital calipers Mitutoyo Digimatic caliper brands (Adhirajan *et al.*, 2003).

RESULTS AND DISCUSSION

Ethanol extraction of fenugreek

The material used in this study was fenugreek seed obtained from Research Center for Medicinal Plant Development, Tawangmangu, Central Java. The fenugreek was crushed to a powder using a blender. This was done to increase the surface area of the simplisia so that contact point between the fenugreek and solvent became larger (Purwantini, 2008). The method used in this study was soxhlet extraction since it had one advantage which the solvent could be recovered after the extraction process was completed. The result of using soxhlet extraction has a high level of purity because the tools sequence made the process effective. However, the limitation of this technique was that the solvent used had to be volatile and should only be used for compounds with high boiling point (Safitri, 2012). One hundred gram samples of fenugreek (which has been mashed) extracted with 350 ml of 98% ethanol yielded in 99.9 ml of ethanol extract fenugreek. This was then concentrated by rotary evaporator to produce 5.68 mg fenugreek seed extract. The extract was in the form of a dark brown viscous liquid, pungent, and slightly bitter taste.

Fenugreek extract phytochemical test

Phytochemical test results show that the fenugreek extract contained saponin, alkaloids, tannins, flavonoids, triterpenoids, steroids and glycosides. Phytochemical examination by Balittro Bogor on December 21, 2012 showed that the fenugreek extract contained saponins, alkaloids, tannins, flavonoids, triterpenoids, steroids and glycosides. One possible explanation for this was that the synthesis of fenugreek seed extract was different from the 95% ethanol extract of fenugreek seeds. Phytochemical test results fenugreek extract can be seen in Table 3 and it showed that the extract contained saponins, alkaloids, tannins, flavonoids, triterpenoids, steroids and glycosides.

Table 3. Phytochemistry screening of The Fenugreek Seed Extract

Compunds in the Fenugreek seeds	Presence
Alkaloid	+
Saponin	+
Flavonoid	+
Triterpenoid	+
Tanin	+
Glikosida	+

Evaluation of hair tonic

Results of organoleptic evaluation and physiochemical properties of the hair tonic by the addition of fenugreek extract 2.5%, 5%, 10% showed homogeneous results. But there was a difference in color between each hair tonic. Formula A (fenugreek 2.5%) are yellow, the formula B (fenugreek 5%) was yellowish brown, while the formula C (klabet 10 was brown. The greater the concentration of extract used, the browner it was. This was influenced by the color of the ethanol of the fenugreek seed extract. Homogeneity test showed all hair tonics were homogeneous, and were also easily dropped and spread on the skin. Fenugreek extract pH was 5.35.pHof formula K2, 5%, K5 and K10%% were 6.07, 6.11 and 6.17 respectively. The pH of hair tonic with minoxidil pH was 6.08. This acidity is in a good pH range for normal human skin condition, which is 4.5 to 6.5 / pH *balance* (Tranggono *et al.*, 2007).

Hair growth activity test

a. Long Hair

Table 4 shows that in the first week, the normal control showed a very similar result with the negative control while K2.5%, K5%, K10%, and minoxidil did not show any difference. However, a significant hair length difference between both the normal control and negative control and hairs in areas treated with K2.5%, K5%, K10%, and minoxidil. Results on the second week showed that when the normal control and negative control was compared, as well as comparing the K2.5% and K5%, no significant difference was found. The hair growth shown by areas treated with K10% and minoxidil, when compared, also did not differ significantly. However, the growth activity shown by normal control and negative control, K2.5% and K5%, and K10% and minoxidil were compared, a significant difference was recorded. In the third week, it was seen that, when compared, both the normal control and the negative control as well as K10% and minoxidil, the difference among these groups was insignificant. K2.5% and K5%, however, still showed a significant difference when compared. A significant difference was also seen when the growth activity shown by normal control and negative control, K2.5% and K5%, and K10% and minoxidil was compared.

Table 4. Hair tonic activity test against long hair

Test Groups	Treatment	Average Lenght (mm) ±SD		
		7^{th} Day	14^{th} Day	21^{st} Day
Rabbit A-F	Normal control	4,26 ±0,14	7,81±0,11	12,10 ±0,27
Rabbit A-F	Negative control (placebo)	4,28 ±1,93	7,59 ±0,07	12,08 ±0,16
Rabbit A-F	Formula K2,5%	5,27 ±2,36	11,18 ±0,19	15,33 ±0,14
Rabbit A-F	Formula K5%	5,16 ±2,32	11,98 ±0,31	18,83 ±0,21
Rabbit A-F	Formula K10%	5,21 ±2,34	15,62 ±0,35	22,72 ±0,20
Rabbit A-F	Positive control(Minoxidil)	5,31 ±2,38	16,69 ±0,26	22,79 ±0,21

Statistical calculation in the third week showed that the data was normally distributed and homogeneous, and thus SPSS test was run. It was shown that there was a significant difference between groups ($p < 0,05$). Based on the results of the hair length on day-21, the hair growth activity shown by K10% was shown to approach the positive control / minoxidil. One study shows that hair tonic containing Emblica officinalis, Bacopa, monnieri, Trigonella foenum-graecum / fenugreek extract and Murraya Koenigii increased the hair growth activity of mice, in which the fenugreek extract concentration used was 2.5%, 5% and 7.5% (Lipi *et al.*, 2007). Another study done using hair tonic containing a combination of tea leaves and *Polyscias scutellaria* leaf extract with a ratio of 2:1 have been shown to improve the hair growth of a rabbit up until 21.76 mm (Purwantini, 2008; Sholikhah and Naniek 2009). Thus, it was shown that the hair tonic used in this study, although using single formula (10% fenugreek seed extract), demonstrated a more significant hair growth activity of 22.72 mm.

b. The Hair Growth Solubilizer Test

To determine the effect of solubilizer *(PPG-26 Buteth 26 and PEG-40 castor oil hidrogenated & water)* on hair growth, the hair tonic was applied to the skin without the addition of any fenugreek extract. From Table 5, the hair length in the first, second, and third week of each formula did not significantly

differ from the average hair length of the normal control. Thus, the solubilizer (Buteth 26 PPG-26 and PPG-40 *hidrogenated castor oil)* did not significantly affect hair growth. *Castor oil* on hair lotion preparations at a concentration of 35% for 1 month does have hair-growing effect. However, the castor oil concentration used in this study was much lower compared to the concentration needed to induce hair growth (35%) (Rusu *et al.*, 2008).

more clearly in the third week compared with the week of unity, probably due to the presence of oil layer.

e. Weight of the hair

In Table 7 it can be seen that in the third week, a greater addition of fenugreek extract corresponded to a greater weight

Table 5. The Average Hair Length Assessed by Solibilizer Activity Test

Test Groups	Treatment	Average Lenght (mm) ±SD		
		7th Day	14th Day	21st Day
Rabbit A-F	Normalcontrol	4,25 ± 0,2006	7,78 ± 0,1391	12,14 ± 0,5432
Rabbit A-F	Formula S 3,1%	4,24 ± 0,1509	7,52 ± 0,2480	12,03 ± 0,7024
Rabbit A-F	Formula S 6,2%	4,21 ± 0,1179	7,48 ± 0,1516	12,05± 0,8163
Rabbit A-F	Formula S 11,2%	4,22 ± 0,2097	7,47 ± 0,1344	12,02 ± 0,5831

Table 6. The average hair diameter measured using micrometer microscope

Test Groups	Treatment	Average Diameter (µm) ±SD		
		7th Day	14th Day	21st Day
Rabbit A-F	Normal control	7,59±0,07	9,23±0,33	12,69±0,15
Rabbit A-F	Negative control (placebo)	8,74 ±0,13	8,24 ±0,52	12,33±0,14
Rabbit A-F	Formula K2,5%	8,16 ±0,15	11,65 ±0,24	15,35 ±0,18
Rabbit A-F	Formula K5%	8,17 ±0,11	12,12 ±0,15	18,89 ±0,27
Rabbit A-F	Formula K10%	8,26 ±0,22	18,78 ±0,14	19,15 ±0,16
Rabbit A-F	Positive control(Minoxidil)	8,69 ±0,13	18,81±0,19	19,13 ±0,33

Table 7. Average weight of the hair

Rabbit	Weight of the hair (mg) on 3rd week of the treatment					
	1	2	3	4	5	6
Rabbit A	145,6	159,3	170,3	187,7	232,8	234,9
Rabbit B	166,2	168,1	178,8	198,1	238,4	235,8
Rabbit C	157,8	162,7	176,4	186,3	226,9	234,6
Rabbit D	143,2	166,7	176,2	186,2	213,6	237,5
Rabbit E	139,8	157,8	176,1	186,5	231,5	244,3
Rabbit F	137,1	141,6	173,9	193,4	226,9	242,2
Average	148,28	159,37	175,28	189,70	228,35	238,22

c. Hair diameter measurement (microscope with micrometer)

The diameter of the first week is shown in Table 6. The normal control did not differ with the negative control. K 2,5%, K5%, K10% and minoxidil showed no difference. There was a significant difference between the normal control, negative, K2,5%, K5%, K10% and minoxidil. Results of the second week showed that there was no significant difference between the normal control sand the negative control. Similarly, the results of K2,5%, K5% also did not differ significantly. Minoxidil and K10% also showed no difference. There are significant differences between the normal control, the negative control, K2,5%, K5%, K10% and minoxidil. In the third week, the normal control did not differ with the negative control. K2,5% was significantly different from the others. K5%, K10% and minoxidil showed no significant difference. Diameter measurements in this study were done using a micrometer microscope and SEM (Scanning Microscope electron). Results of both methods were shown to be similar.

d. Hair morphology examination using SEM (Scanning Microscope electron)

Figure 1 through 8 show the morphology of hair as well as hair diameter measurements using SEM (Scanning Electrone Microscope). Hair cuticle looked in complete and smaller in the first week compared to the second and third weeks (looks like a roof tile arrangement). Visible light reflection was seen of the rabbit hair. The weight of the rabbit hair in the third week in the normal control, negativecontrolandK2, 5% were not significantly different. The hair weight onK5% weight significantly differed to other treatments. The hair weight on K10% did not differ significantly with weight of that treated with minoxidil.

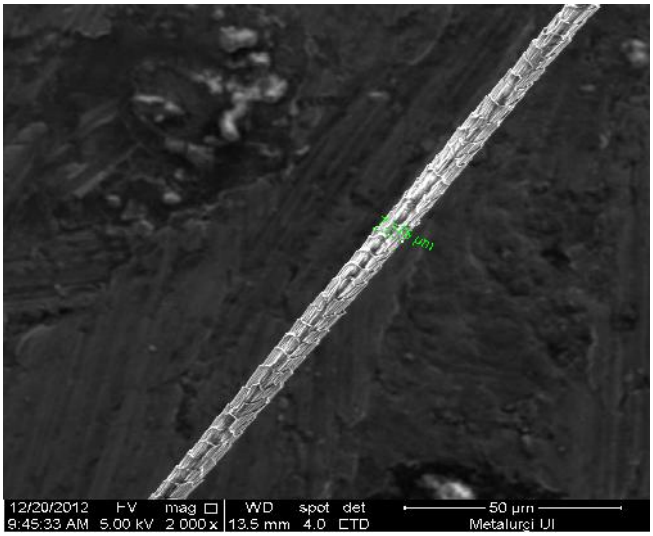

Figure 1. Normal control- First week

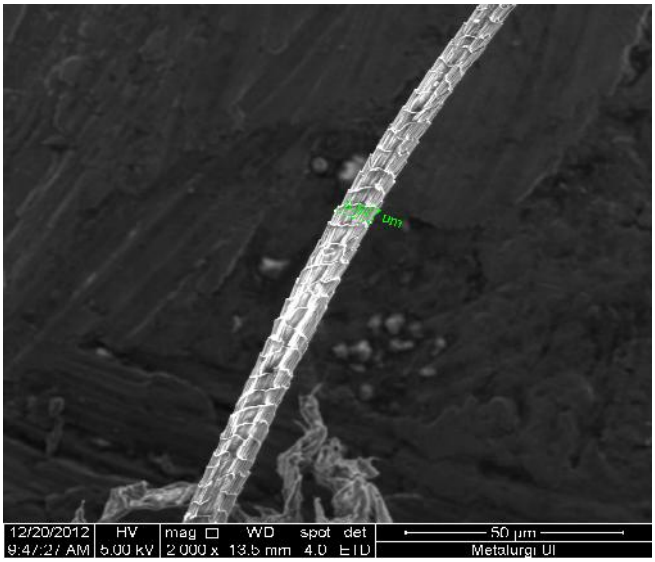

Figure 2. Positive control- First week

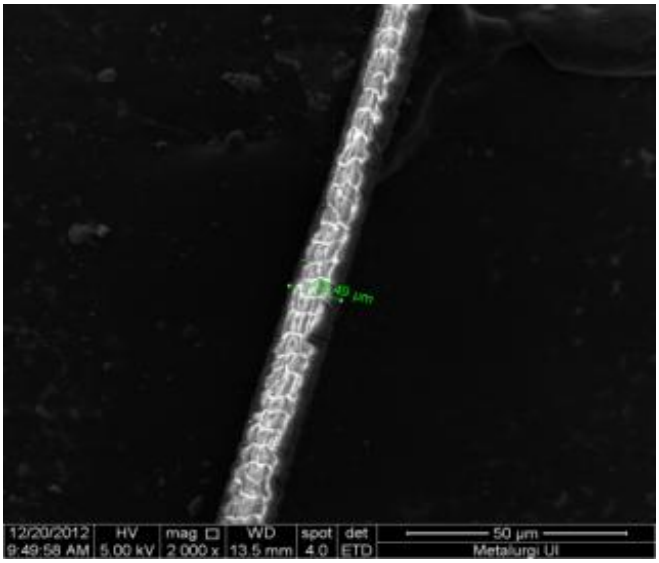

Figure 5. Negative control- Third week

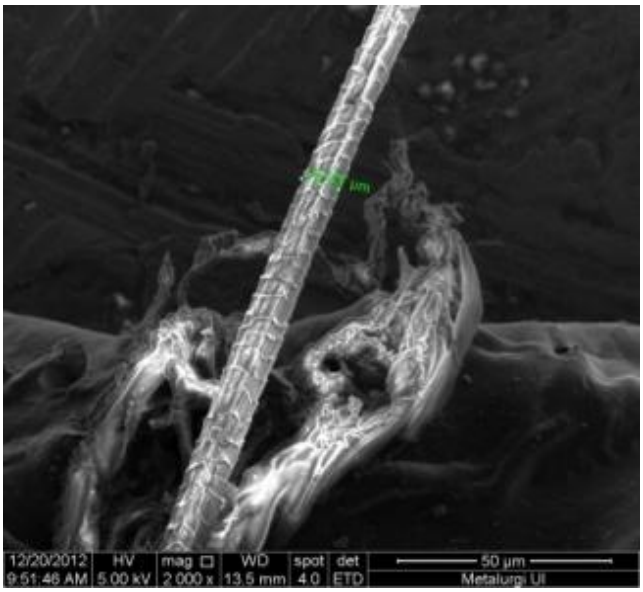

Figure 3. Normal control- Second week

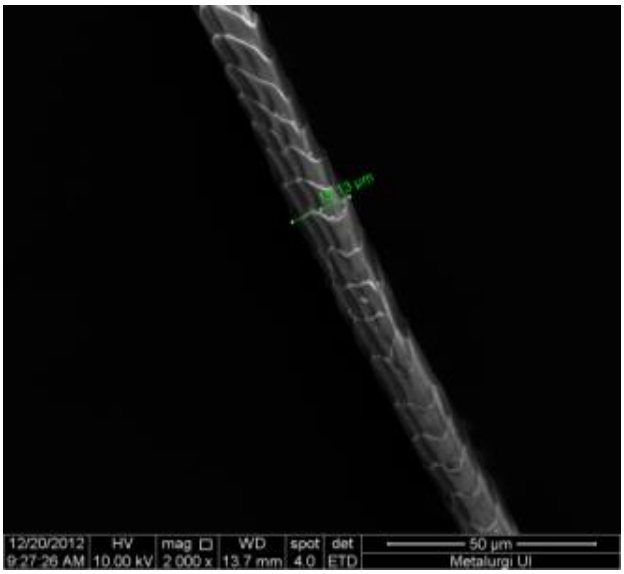

Figure 6. K2,5% - Third week

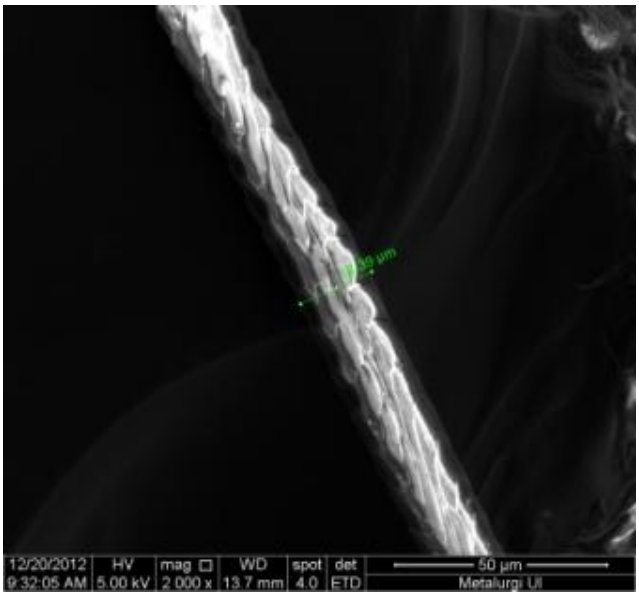

Figure 4. K10% -Second week

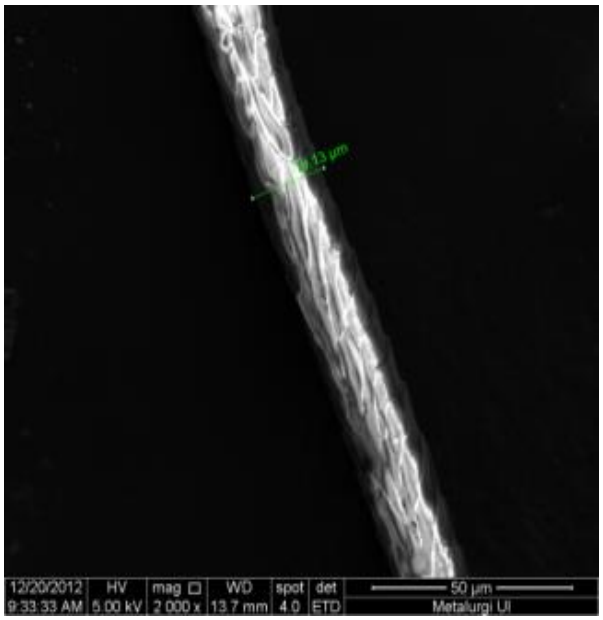

Figure 7. K10%- Third week

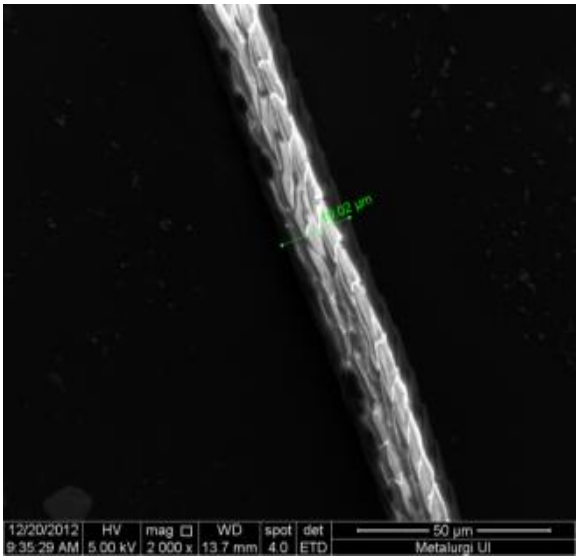

Figure 8. Positive control- Third week

Draize eye test test

The sensitivity assessment test was conducted to determine the safety of the fenugreek extract. The test was performed on3rabbitstodetermine the ocular sensitivity index. Tests were performed on the left eye with the controls on the right eye. 3 drops of sterile 2.5% fenugreek extract in physiological saline was applied to the rabbit's left eye, with observations on 30 minutes, 60 minutes, 120 minutes, 240 minutes, 1 day, 2 days, 3 days and 4 days. The left eye did not show a picture of opacity, normal conjunctiva, no swelling eye lid and the iris picture looked normal. The eyes were shown to produce tears. The ocular irritation index on the first day was 2 and on the second, third and fourth day it was 0 (maximum ocular irritation index of 110). Thus, it can be concluded that fenugreek extracts gave mild irritation.

Conclusion

Fenugreek seed extract has a positive effect on hair growth process compared to placebo, with an optimal concentration of10%. Sensitivity test (Draize skin test) of the fenugreek seed extract on the skin did not cause sensitivity and sensitivity test (Draize eye test) showed mild sensitivity effect.

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