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Effects of Tocotrienol Supplementation on Hair Growth in Human Volunteers

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Abstrak: Kajian telah menunjukkan bahawa terdapat perhubungan di antara oksidatif stres dengan keguguran rambut. Pesakit yang mengalami keguguran rambut secara amnya mempamerkan paras anti-oksidan yang lebih rendah pada kawasan kulit kepala serta index peroksidan lemak yang lebih tinggi. Tokotrienol merupakan vitamin E dan mempunyai efek anti-oksidan yang tinggi. Oleh itu, satu kajian telah dijalankan untuk mengkaji kesan suplementasi tokotrienol ke atas sukarelawan yang mengalami masalah keguguran rambut. Dua puluh satu sukarelawan diagihkan secara rawak untuk menerima 100 mg tokotrienol campuran setiap hari secara oral manakala 17 sukarelawan diagihkan untuk menerima placebo secara oral. Sukarelawan-sukarelawan tersebut dipantau pada bulan ke-0 (sebelum menerima suplementasi tokotrienol), ke-4 dan ke-8 bagi menentukan bilangan rambut pada kawasan kepala yang telah ditentukan terlebih dahulu serta berat 20 helai keratan rambut sepanjang 1 cm. Sukarelawan-sukarelawan yang menerima suplementasi tokotrienol menunjukkan peningkatan bilangan rambut yang signifikan berbanding kumpulan placebo. Kumpulan suplementasi tokotrienol mencatatkan peningkatan sebanyak 34.5% pada penghujung bulan ke-8 berbanding dengan penurunan sebanyak 0.1% yang dicatatkan oleh kumpulan placebo. Namum demikian, berat 20 helai keratin rambut tidak banyak berbeza di antara kedua-dua kumpulan pada penghujung tempoh kajian. Sebagai kesimpulan, kajian ini menunjukkan bahawa suplementasi dengan kapsul tokotrienol meningkatkan bilangan rambut pada sukarelawan yang mengalami masalah keguguran rambut berbanding dengan kumpulan placebo. Kesan ini berkemungkinan disebabkan oleh aktiviti anti-oksidan tokotrienol yang membantu menurunkan peroksidan lemak dan oksidatif stres pada kulit kepala, dimana kedua-duanya telah dihubungkaitkan dengan keguguran rambut.

Kata kunci: Pertumbuhan Rambut, Tokotrienol, Anti-oksidan

Abstract: Studies have shown an association between oxidative stress and alopecia. Patients with alopecia generally exhibit lower levels of antioxidants in their scalp area as well as a higher lipid peroxidation index. Tocotrienols belong to the vitamin E family and are known to be potent antioxidants. Hence, a study was conducted to investigate the effect of tocotrienol supplementation on hair growth in volunteers suffering from hair loss. Twenty one volunteers were randomly assigned to orally receive 100 mg of mixed tocotrienols daily while 17 volunteers were assigned to receive placebo capsule orally. The volunteers were monitored for the number of hairs in a pre-determined scalp area as well as the weight of 20 strands of 1 cm length hair clippings at 0 (before supplementation), 4 and 8 months. The number of hairs of the volunteers in the tocotrienol supplementation group increased significantly as compared to the placebo group, with the former recording a 34.5% increase at the end of the 8-month supplementation as compared to a 0.1% decrease for the latter. Nevertheless, the cumulative weight of 20 strands of hair clippings

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did not differ much from the baseline for both supplementation groups at the end of the study period. In conclusion, this trial demonstrated that supplementation with tocotrienol capsules increases hair number in volunteers suffering from hair loss as compared to the placebo group. This observed effect was most likely to be due to the antioxidant activity of tocotrienols that helped to reduce lipid peroxidation and oxidative stress in the scalp, which are reported to be associated with alopecia.

Keywords: Hair Growth, Tocotrienols, Antioxidants

INTRODUCTION

Scalp hair plays an important function in humans. In addition to providing cranial cushioning and shielding the scalp from direct sun rays, hair has sociological meanings in terms of gender, age, values and status (Cash 2001). Scalp hair is also a vital aspect of an individual's physical appearance and helps to project a positive image (Cash 2001). Hair loss and balding, on the other hand, are associated with negative attributes (Rushton ***et al***. 2002). Hair loss or alopecia is a common problem suffered by both sexes and has been reported to cause significant psychological effects, such as diminished self-esteem, emotional distress, embarrassment and social inadequacy, with these detrimental effects being more significant in women (Cash ***et al.*** 1993). Van der Donk ***et al.*** (1994) observed that alopecia could severely affect the quality of life of the majority of the sufferers, whereby 88% of sufferers experienced negative effects in their daily life and 75% and 50% encountered negative self-esteem and social problems, respectively.

There are many types of alopecia, such as androgenetic alopecia (AGA), alopecia areata, telogen effluvium, hair loss due to systemic medical problems such as thyroid disease and adverse drug effects, as well as hair loss due to scalp or hair trauma, discoid lupus erythematosus, lichen planus and structural shaft abnormalities, to name a few (Hogan & Chamberlain 2000). The causes for the above are numerous and vary between the different types of alopecia. In most instances, the aetiology is still unknown. However, one of the universal causes of increased hair thinning or shedding is nutritional deficiency. It was previously reported that children with protein malnutrition would exhibit fragile and finer hairs that easily fall or break off and display a lower daily rate of growth (Bradfield & Bailey 1969; Sims 1968). Nutritional factors that have been identified to be essential in preventing hair loss include iron and an essential amino acid, L-lysine (Rushton ***et al.*** 1990; Rushton 2002). Rushton ***et al.*** (2002) reported that women with chronic telogen effluvium have low serum ferritin levels and proceeded to demonstrate that daily supplementation of iron and L-lysine for 6 months could cause significant increases in hair numbers.

Recently, Naziroglu and Kokcam (2000) showed that there was an association between oxidative stress and alopecia. They reported that the levels of reduced glutathione (GSH) and activities of glutathione peroxidase (GSH-Px), which are present to protect against damages caused by reactive oxygen species such as free radicals and peroxides, were significantly lower in patients with alopecia than in controls, whereas the levels of thiobarbituric acid reactive

substances (TBARS), which indicate lipid peroxidation and oxidative stress, were significantly higher.

Tocotrienols together with tocopherols are members of the vitamin E family. They share similar structural features of a chroman head and a 16-carbon phytyl chain. The structural difference between them lies mainly in the latter possessing a saturated phytyl chain whereas that of the former possesses three unsaturated double bonds (Papas 1999; Theriault ***et al.*** 1999). Tocotrienols also possess more potent antioxidant property, and Serbinova ***et al.*** (1991) showed that *α*-tocotrienol was 40- to 60-fold more potent than *α*-tocopherol against lipid peroxidation in rat liver microsomal membranes. This superiority has been ascribed to the ability of tocotrienols to better distribute within the fatty layers of the cell membranes and hence permit better interaction with lipid radicals. Moreover, tocotrienols have been shown to afford protection to the skin against UV light- and ozone-induced oxidative stress (Traber ***et al.*** 1997).

In view of the association between alopecia and oxidative stress and the high antioxidant potency of the tocotrienols, a study was thus performed in volunteers with alopecia to evaluate the efficacy of tocotrienol supplementation in improving hair coverage of the scalp and preventing hair thinning in patients suffering from alopecia.

MATERIALS AND METHODS

Patient Population

Thirty eight male and female volunteers ranging from 18 to 60 years old who met the inclusion criteria were recruited into the trial. Volunteers had varying levels of hair loss, ranging from patchy loss of scalp hair to more severe loss of scalp hair. Hair loss must have been present for at least 2 months and the alopecia area could not have any visual evidence of new hair growth. All volunteers were in good general health with no evidence of systemic illness including cardiac, psychiatric, thyroid and scalp diseases.

Volunteers were excluded from the study if they had known hypersensitivity to tocotrienols or if they had undergone hair restoration procedures, consumed hair growth medications or applied hair enhancement products in the past 6 months. Patients who had undergone chemotherapy and experienced scalp trauma were also excluded. Concomitant use of medications, such as steroids, oral contraceptives, cytotoxic agents, vasodilators, antihypertensive agents, anti-convulsant drugs, *ß*-adrenergic receptor blockers, diuretics, spironolactone, cimetidine, cyclosporine or ketoconazole, during the 6 months prior to the study was also not allowed. Patients on iron and vitamin B12 supplementation were also excluded.

Volunteers were also instructed not to alter their hairstyle, the hair care products (such as shampoo or conditioner) currently in use or dye their hair during the study period.

Study Design

This study was an 8 month (32 week), randomised, double-blind, placebo-controlled trial conducted at the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Pinang. The protocol and informed consent form were approved by an institutional review board and written informed consent was obtained from each volunteer prior to enrolment into the study. Thirty eight volunteers participated in the trial, and 21 volunteers were randomly selected to receive tocotrienol supplementation (tocotrienol supplementation group) while 17 were randomly selected to receive the placebo (placebo group). The placebo is a soft gelatine capsule containing 600 mg of soya bean oil (Hovid Sdn Bhd, Ipoh, Malaysia), while tocotrienol supplementation consisted of mixed tocotrienols formulated in a soft gelatine capsule (Hovid Sdn Bhd). Each tocotrienol capsule contained 50 mg of mixed tocotrienols (30.8% α-tocotrienol, 56.4% γ-tocotrienol and 12.8% δ-tocotrienol) as well as 23 IU of α-tocopherol. The volunteers were required to take one capsule of either the placebo or tocotrienol twice daily after meal over a period of 8 months. Hence, the total daily intake of tocotrienols for each volunteer in the treatment group was 100 mg. The capsules were provided on a monthly basis and compliance with the supplementation regimen was monitored by counting the capsules that were left over during the monthly visit. The volunteers were also counselled and advised to take the capsules regularly as per the instruction given to ensure compliance. The volunteers were required to report any side/adverse reactions, additional medication taken and any new diagnosis during the trial period.

Efficacy Evaluation

Two parameters were chosen to evaluate the efficacy of tocotrienol supplementation (Price *et al.* 2002):

1. Hair counts
   Hair counts served as the primary efficacy measure. An area of 2 × 2 cm was selected in the area of hair thinning for each patient in the balding vertex scalp, and the 2 opposing corners of the square were permanently marked (using a 4 cm^2 wire template) to ensure that the hair in same area was counted at each visit. The count was done by an investigator who was blinded to the treatment the volunteers were receiving. The resulting hair counts were used to calculate the percentage change from baseline.
2. Weight of hairs
   A small tuft of hair (at least 20 strands) within the demarcated area was clipped horizontally. Twenty strands were randomly chosen and cut to 1 cm in length. The total weight was obtained using a microbalance and the resulting mean weight was used to calculate the percentage change from baseline.

Both parameters were obtained at baseline, 4 months (16^{th} week) after the start of the study and at the end of 8 months (32^{nd} week) for efficacy evaluation. Altogether, there were three intervals.

Statistical Calculation

The data collected at baseline and during the supplementation period for both groups were compared using an analysis of variance procedure (ANOVA) for a two-factor repeated measures split-plot experimental design (Kirk 1968). If a statistically significant difference for the main effect of interval was observed, Tukey's post-hoc test was employed. When significant interaction between supplementation and an interval was observed, simple main effect analysis (pairwise comparison) was employed to locate the pair that gave rise to the observed difference. The homogeneity of the baseline characteristics between the two groups, namely the number and weight of hairs, was also assessed. A statistically significant difference was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Thirty eight volunteers with signs of hair loss or balding were enrolled in the study, with the supplementation group having 21 volunteers (19 male and 2 female) and the placebo group (controlled group) having 17 volunteers (all male). The age of the study population ranged from 18 to 59 years. A wide range in the age of volunteers was observed because hair loss is known to affect a wide range of the population irrespective of age. Although it is more commonly seen with increasing age, young adults are also affected. Moreover, hair loss may be attributed to universal causes such as nutritional deficiency and oxidative stress, which can affect a wide age group. The volunteers had hair loss problem for approximately 2 to 5 years.

Comparability of the treatment groups with respect to initial hair counts as well as the weight of hair prior to study commencement was also assessed. The mean number of hairs of volunteers in the placebo group at baseline was 289.0 ± 98.3, while that of the tocotrienol supplementation group was 284.8 ± 111.3. As for the mean weight of 20 strands of hair at baseline for the placebo and tocotrienol supplementation groups, the values were 0.1002 ± 0.0639 g and 0.0920 ± 0.0565 g, respectively. The values between the placebo and supplementation groups were comparable for both parameters and no statistically significant difference ($p > 0.05$) was detected.

Thirty five volunteers completed the entire study and were included in the evaluation of the efficacy of tocotrienol supplementation. Three volunteers were lost to follow-up, one (female) from the tocotrienol supplementation group and two from the placebo group. Compliance with the supplementation regimen was satisfactory, as evidenced by the capsule counts during their monthly visits. No severe drug reaction related to the administration of the drug product and placebo was recorded, thus indicating that long-term administration of 100 mg of tocotrienols for up to 8 months was tolerable. The compliance with the study protocol was also deemed satisfactory and no protocol deviation was recorded.

Table 1 shows the hair numbers at baseline and at 4 and 8 months from baseline for volunteers receiving placebo and tocotrienol supplementation together with the percentage change from baseline. It is evident that volunteers in the tocotrienol supplementation group showed a gradual mean increase in hair

numbers from baseline up to the 8th month interval with a mean percentage increase of more than 34% at the end of the study. In comparison, the number of hairs for volunteers in the placebo group did not show any appreciable increase at the end of the 8 months study. A negligible percentage change of 0.1% was recorded by this group.

Table 1: Mean numbers of hairs at baseline and after 4 and 8 months of tocotrienol and placebo supplementation (mean ± SD, percentage change from baseline).

Supplementation	Intervals		
	Baseline	4 months	8 months
Tocotrienol supplementation	284.8 ± 111.3	328.0 ± 121.1 (15.2%)	383.1 ± 120.9 (34.5%)
Placebo	289.0 ± 98.3	298.2 ± 92.4 (3.2%)	288.7 ± 89.9 (–0.1%)

There was no statistically significant difference in the main effect of supplementation but a significant main effect of interval was evident. Tukey's post-hoc test showed that the hair numbers at the 8 month interval were significantly greater than those at baseline and at the 4 month interval. Subsequent simple main effect analysis indicated that a statistically significant difference occurred between baseline and post-supplementation at the 8 month interval for volunteers supplemented with tocotrienols only. All other pairwise comparisons (baseline vs. 4 month interval for placebo and supplementation group and baseline vs. 8 month interval for placebo group) did not show statistically significant differences. This indicates that there was no placebo effect and the increase in the number of hairs observed in the volunteers could be ascribed to tocotrienol supplementation.

Pairwise comparison also demonstrated that the hair numbers of volunteers of the tocotrienol supplementation and placebo groups were significantly different at the 8 month interval. At this interval, the number of hairs was much higher for the tocotrienol supplementation group volunteers than those in the placebo group. Nevertheless, the same could not be ascribed for observations obtained at the 4 month interval, whereby no statistically significant difference was observed between the 2 groups at this interval. In this regard, it can be summarised that tocotrienol supplementation for a period of 8 months resulted in significant increases in the numbers of hairs as compared to that in the placebo group.

All volunteers with the exception of one in the tocotrienol supplementation group showed a positive response at the end of the 8 month study, recording an increase in the number of hairs in the area of scalp evaluated. Eight volunteers (40.0%) showed hair increases of more than 50%, 1 volunteer (5.0%) had a 25% to 50% increase, 9 volunteers (45.0%) had increases between 10% to 25% while 1 volunteer (5.0%) showed a hair increase of less than 10%. Only 1 volunteer (5.0%) in the tocotrienol supplementation group had a slight decrease in the number of hairs. On the other hand, only 8 volunteers in the placebo group showed an increase in the number of hairs after

8 months, with 1 (6.7%) showing more than 20% increase and the remaining 7 (46.7%) showing negligible increases. Seven volunteers (46.7%) had a decrease in the number of hairs.

However, there was no statistically significant increase in the weight of hair ($p > 0.05$) between pre- and post-supplementation for both groups of volunteers (tocotrienol supplementation and placebo group). The mean percentage of weight increment after 8 months of supplementation was 5.9% in the tocotrienol supplementation group, while that of the placebo group had a slight decrease of 2.0% (Table 2).

Table 2: Mean weight (g) of 20 strands of hair at baseline and after 4 and 8 months of tocotrienol and placebo supplementation (Mean ± SD, percentage change from baseline).

Supplementation	Intervals		
	Baseline	4 months	8 months
Tocotrienol supplementation	0.0929 ± 0.0565	0.0935 ± 0.0590 (0.6%)	0.0984 ± 0.0680 (5.9%)
Placebo	0.1002 ± 0.0639	0.0975 ± 0.0547 (–2.7%)	0.0982 ± 0.0563 (–2.0%)

Recently, Naziroglu and Kokcam (2000) showed that an association existed between oxidative stress and alopecia. Specifically, the levels of GSH and activities of GSH-Px were significantly lower in patients with alopecia than in controls, whereas TBARS levels, which indicate lipid peroxidation and oxidative stress, were significantly higher. Similar observations were also reported by Koca *et al.* (2005) and Akar *et al.* (2002), whereby the authors in the former observed an increased lipid peroxidation and a decrease in superoxide dismutase (SOD) levels in patients with alopecia areata compared to controls. In the latter, TBARS levels in the scalp of patients with alopecia areata were higher than those of controls, although the authors reported a higher levels of SOD and GSH-Px in alopecia patients. Girat *et al.* (1996) similarly reported that TBARS levels increased 2-fold and glutathione content was reduced 2.5-fold in sebaceous gland-enriched scalp skin of men affected by male pattern baldness as compared to the controls. Moreover, Naziroglu and Kokcam (2000) demonstrated that both the *ß*-carotene and *α*-tocopherol levels in plasma were lower in patients with alopecia, albeit the latter was not statistically significant. This led to the authors' postulation that antioxidant treatment such as using *ß*-carotene, vitamin E and selenium may benefit patients with alopecia. Hence, the positive effect of tocotrienol supplementation in the current study could most likely be ascribed to its potent anti-oxidant activities.

Nevertheless, other mechanisms merit further investigations such as those that play a role in AGA. It has been shown by Hee (2008) that incubation of a synthetic androgen with rat vibrissae dermal papilla cells (DP6) led to increased production of reactive oxygen species (ROS) intracellularly. In addition, the authors also reported that androgen-inducible transforming growth factor beta 1 (TGF-*ß*1), a key mediator in the formation of AGA, was mediated by ROS and could be prevented by antioxidants or ROS inhibitors in the hair follicle dermal

papilla cells (Hee 2008; Inui ***et al.*** 2003). The above observations led the authors to suggest that antioxidants could potentially be used to control androgen-mediated pattern hair loss.

CONCLUSION

In conclusion, this trial demonstrated that supplementation with tocotrienol capsules increases hair numbers in volunteers suffering from hair loss as compared to the placebo group. A possible explanation for the effects could be due to the potent antioxidant activity of tocotrienols that help to reduce lipid peroxidation and oxidative stress in the scalp, which are known to be associated with alopecia.

REFERENCES

Akar A, Arca E, Erbil H, Akay C, Sayal A and Gur A R. (2002). Antioxidant enzymes and lipid peroxidation in the scalp of patients with alopecia areata. *Journal of Dermatological Science* 29(2): 85–90.

Bradfield R B and Bailey M A. (1969). Hair root response to protein malnutrition. In W Montagna and R L Dobson (eds.). *Biology of skin, hair growth,* vol 9: 7. Oxford: Pergamon Press, 109–119.

Cash T F, Price V H and Savin R C. (1993). Psychological effects of androgenetic alopecia on women: Comparisons with balding men and with female control subjects. *Journal of the American Academy of Dermatology* 29(4): 568–575.

Cash T F. (2001). The psychology of hair loss and its implications for patient care. *Clinics in Dermatology* 19(2): 161–166.

Girat M, Cervello I, Nogues M R, Puerto A M, Ortin F, Argany N and Mallol J. (1996). Glutathione, glutathione S-transferase and reactive oxygen species of human scalp sebaceous glands in male pattern baldness. *Journal of Investigative Dermatology* 107(2): 154–158.

Hee C E. (2008). The involvement of ROS on androgen inducible TGF beta 1 regulation derived from dermal papilla cells; a suggestive implication of ROS on androgenetic alopecia. *Journal of the American Academy of Dermatology* 58(2) Suppl 2: AB84.

Hogan D J and Chamberlain M. (2000). Male pattern baldness. *Southern Medical Journal* 93(7): 657–662.

Inui S, Fukuzato Y, Nakajima T, Yoshikawa K and Itami S. (2003). Identification of androgen-inducible TGF-*β1* derived from dermal papilla cells as key mediator in androgebetic alopecia. *Journal of Investigative Dermatology: Symposium Proceedings* 8(1): 69–71.

Kirk R E. (1968). *Experimental design: Procedure for the behavioural science*. CA, USA: Brooks/Cole Publishing Company.

Koca R, Armutcu F, Altinyazar H and Gurel A. (2005). Evaluation of lipid peroxidation, oxidant/antioxidant status and serum nitric oxide levels in alopecia areata. *Medical Science Monitor* 11(6): 296–299.

Naziroglu M and Kokcam I. (2000). Antioxidants and lipid peroxidation status in the blood of patients with alopecia. *Cell Biochemistry and Function* 18(3): 169–173.

Papas A M. (1999). Vitamin E: Tocopherols and tocotrienols. In A M Papas (ed.). *Antioxidant status, diet, nutrition and health.* Boca Raton, Fl: CRC Press, 189–210.

Price V H, Menefee E, Sanchez M, Ruane P and Kaufman K D. (2002). Changes in hair weight and hair count in men with androgenetic alopecia after treatment with finasteride, 1 mg daily. *Journal of the American Academy of Dermatology* 46(4): 517–523.

Rushton D H, Norris M J, Dover R and Busuttil N. (2002). Causes of hair loss and the developments in hair rejuvenation. *International Journal of Cosmetic Science* 24(1): 17–23.

Rushton D H, Ramsay I D, James K C, Norris M J and Gilkes J J H. (1990). Biochemical and trichological characterization of diffuse alopecia in women. *British Journal of Dermatology* 123(2): 187–197.

Rushton D H. (2002). Nutritional factors and hair loss. *Clinical and Experimental Dermatology* 27(5): 396–404.

Serbinova E, Kagan V, Han D and Packer L. (1991). Free radical recycling and intramembrane mobility in the antioxidant properties of alpha tocopherol and alpha tocotrienol. *Free Radical Biology and Medicine* 10(5): 263–275.

Sims R T. (1968). The measurement of hair growth as an index of protein synthesis in malnutrition. *British Journal of Nutrition* 22: 229–236.

Theriault A, Chao J T, Wang Q, Gapor A and Adeli K. (1999). Tocotrienol: A review of its therapeutic potential. *Clinical Biochemistry* 32(5): 309–319.

Traber M G, Podda M, Weber C, Thiele J, Rallis M and Packer L. (1997). Diet-derived and topically applied tocotrienols accumulative in skin and protect the tissue against ultraviolet light-induced oxidative stress. *Asia Pacific journal of Clinical Nutrition* 6(1): 63–67.

Van der Donk J, Hunfeld J A M, Passchier J, Knegt-Junk K J and Nieboer C. (1994). Quality of life and maladjustment associated with hair loss in women with alopecia androgenetica. *Social Science & Medicine* 38(1): 159–163.