RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2012.38.262

ScienceAsia 38 (2012): **262–267**

Sebumetric and mexametric evaluation of a fennel based cream

Akhtar Rasul[a,b,*], **Naveed Akhtar**[a], **Muhammad Iqbal**[b], **Barkat Ali Khan**[a], **Asadullah Madni**[a], **Ghulam Murtaza**[c], **Muhammad Khurram Waqas**[a], **Tariq Mahmood**[a]

[a] Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur 63100 Pakistan

[b] School of Pharmacy, The University of Faisalabad, Faisalabad 37610 Pakistan

[c] Department of Pharmacy, COMSATS Institute of Information Technology, Abbotabad, Pakistan

*Corresponding author, e-mail: dr.akhtarstar@gmail.com

Received 24 Dec 2011
Accepted 1 May 2012

ABSTRACT: This study aims to investigate the effects of a topical cream (emulsion) containing 4% extract of fennel (*Foeniculum vulgare*) on functional skin parameters like sebum content, skin melanin, and skin erythaema, using the base without fennel as a control. Fennel extract was entrapped in the inner aqueous phase of emulsion. The creams were applied to 11 healthy male volunteers for a period of 12 weeks. Skin parameters were measured fortnightly using a Mexameter MPA 5 and a Sebumeter MPA 5 to determine the effects produced. The base showed insignificant ($p > 0.05$) increase while the active formulation showed significant ($p \leqslant 0.05$) decrease in skin melanin and sebum content, which can be attributed to the presence of linoleic acid and oleic acids in fennel. Analysis using a paired sample t-test showed that the base decreased skin erythaema insignificantly while the formulation exerted a significant decrease indicating that it possessed anti-erythaemic effects. The formulation can therefore be used safely for the treatment of acne and as a skin whitening agent in males.

KEYWORDS: *Foeniculum vulgare*, emulsion, skin whitening, anti erythaemic

INTRODUCTION

Phenolic compounds found commonly in both edible and non-edible plants are vital for human health because of their antioxidant capacity[1]. The importance of the antioxidant compounds to preserve health and to protect from diseases interest scientists, food manufacturers, and consumers seeking purposeful food with precise health effects[2]. Herbal spices, being a promising source of phenolics, flavonoids, anthocyanins, and carotenoids, are usually used to impart flavour and enhance the shelf-life of dishes and processed food products[3]. Essential oils, extracts, and bioactive constituents of several spices and herbs are well known to exert antioxidant and antimicrobial activities[4]. Antioxidants act as free radical scavengers, inhibit lipid peroxidation, and other free radical-mediated processes and are able to protect the human body as well as processed foods from oxidative damage[5].

Currently, the use of plant-based natural antioxidants, such as those containing phenolic substances like flavonoids and phenolic acids and tocopherols in foods, as well as preventive and therapeutic medicine, is gaining much recognition[6]. Plants are appropriate for pharmaceutical research and drug development, because their constituents can be used not only as therapeutic agents but also as starting materials or models for the synthesis of drugs or pharmacologically active compounds[7].

Fennel (*Foeniculum vulgare*) is a plant belonging to the family Apiaceae with a long history of herbal uses. Traditionally, fennel seeds are used as anti-inflammatory, analgesic, carminative, diuretic, and antispasmodic agents[8]. Fennel has also shown antihirsutism activity[9]. Recently, there has been considerable interest in the antioxidant potential and antimicrobial activities of fennel seed extracts and essential oil[10,11].

In this study, a fennel extract emulsion and its base were evaluated for its effects on various functional skin parameters.

MATERIALS AND METHODS

Materials

Ethanol (95%) was purchased from BDH Chemicals, England, Abil-EM90 from Franken Chemicals, Germany, and paraffin oil from Merk KGaA Darmstadt, Germany. Mexameter MPA 5 and Sebumeter MPA 5

were made by Courage Khazaka, Germany. Distilled water was prepared in the laboratory of the Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. Fennel seeds were purchased from a local market, identified, and authenticated by Prof. Dr Muhammad Arshad at Cholistan Institute of Desert studies, The Islamia University of Bahawalpur, and a voucher specimen was preserved (voucher # FV-SD-4-11-20) at the herbarium for future reference.

METHODS

Preparation of the creams

The detailed method of preparation of the creams, i.e., base and formulation, has been previously reported [12] and is briefly described here. *F. vulgare* seeds extract was prepared by maceration. For this purpose, seeds (200 g) were finely ground and extracted at room temperature with 1.0 l of 95% ethanol for 48 h. The filtered extract was evaporated under reduced pressure at 40 °C in a rotary evaporator. The obtained extract was stored at 0 °C. For the formulation, paraffin oil (14%) and surfactant Abil-EM90 (2.5%) were heated together up to 75 ± 1 °C. Simultaneously, distilled water (quantity sufficient to make 100%) was heated at the same temperature, and then extract (4%) was added into it. The aqueous phase was then added to the oily phase drop by drop and stirred with a homogenizer to obtain the creams with reliable consistency. In the case of the base, no extract was added to the aqueous phase. These were kept at different storage conditions of 8 ± 0.1 °C, 25 ± 0.1 °C, 40 ± 0.1 °C, and 40 ± 0.1 °C with 75% relative humidity for a period of 8 weeks to check for stability, and were found stable showing no creaming or phase separation [12].

Skin irritation evaluation

Patch tests were performed on both forearms of every volunteer on the first day of skin testing to assess any skin irritation. A 5 cm $\times$ 4 cm region was marked on the forearms. The patch, prepared from bandage, for the left forearm contained base while the patch for right forearm had formulation in it. Each patch was applied to the marked regions separately on each forearm. The regions were covered with the surgical dressing after application. The patches were removed after 48 h and the forearms were observed for any skin redness/irritation by a physician [13, 14].

Study method

The study was designed single blinded for the comparison of two creams recruiting healthy 11 male volunteers with mean age of 45 years and consent forms were taken. The formulations intended to be tested in this study for effects on various functional skin parameters were two creams (emulsions). One cream was base with no extract (B) and the other was active formulation with extract (F). These were prepared by adding aqueous phase to the oily phase with continuous stirring. Oil phase contained paraffin oil and surfactant (Abil-EM 90), and aqueous phase containing water was heated to 75 ± 1 °C and then extract was added in it. In case of base, no extract was added in the aqueous phase.

The applications of creams were carried out on the cheeks of volunteers. The volunteers were asked to use an amount which can be absorbed easily on the skin. Each volunteer applied creams at night on cheeks for the period of 12 weeks and came for measurement on the 2nd, 4th, 6th, 8th, 10th, and 12th week in the morning at 10 a.m. They were allowed to wash their faces with water and sit to become adapted to the environment for 30 min before any measurements were taken. Values for different parameters were taken in controlled room temperature 25 ± 1 °C.

Ethical standards

This study was approved by the Board of Advanced Studies and Research (BASR), and its Ethical Committee for in vivo Studies (Reference No. 3715/Acad.), The Islamia University of Bahawalpur, and was conducted according to the international guidelines of Helsinki Declaration [15].

Mathematical analysis

The percentage changes for the individual values of skin sebum content, skin erythaema, and melanin of volunteers were calculated by the following formula: Change $= (A-B)/B$, where A is the individual value of any parameter at the 2nd, 4th, 6th, 8th, 10th, or 12th week and B is the initial value (0 h) of that parameter.

Statistical analysis

Paired samples t-test for variation between the two preparations and two-way ANOVA for variation between different times intervals were analysed using SPSS 12.0 on computer using a 5% level of significance.

RESULTS AND DISCUSSION

Skin erythaema

In this study, it was found that both base and the active formulation showed continuous decline in skin erythaema values throughout the study period of

Table 1 Values of skin erythaema at different time intervals after application of base (B) and formulation (F).

Volunteer #	0 h		Week 2		Week 4		Week 6		Week 8		Week 10		Week 12	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	312	304	310	310	306	312	301	301	299	297	295	289	287	283
2	454	465	446	470	441	474	438	464	443	457	440	453	423	448
3	352	345	346	340	345	344	336	339	325	330	321	327	317	318
4	424	437	421	435	418	430	411	423	417	438	418	422	413	416
5	346	328	334	324	332	313	335	310	327	314	322	311	320	304
6	438	456	435	465	439	455	431	453	430	447	436	444	459	435
7	517	508	521	512	530	505	534	501	537	498	532	490	556	487
8	498	506	487	493	484	492	478	487	477	477	484	464	480	462
9	502	487	498	480	495	476	505	463	512	460	507	466	524	470
10	426	402	430	412	428	415	418	422	423	429	427	406	433	397
11	403	396	409	370	412	363	414	379	420	369	417	391	413	354
Average	425	421	422	419	421	416	418	413	419	411	418	406	420	398
±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
SD	67	72	68	72	70	71	72	71	76	70	77	69	85	72

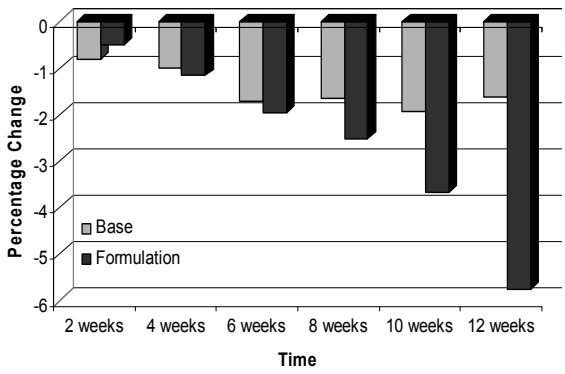

Fig. 1 Percentage of changes in skin erythaema after application of base and formulation.

12 weeks. The values obtained by Mexameter MPA 5 is presented in Table 1.

The Mexameter MPA 5 measures skin erythaema and melanin based on the absorption principle. The special probe of the Mexameter emits light of three defined wavelengths. A receiver measures the light reflected by the skin. The positions of emitter and receiver guarantee that only diffuse and scattered light is measured. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. The melanin is measured by two wavelengths. These wavelengths have been chosen to achieve different absorption rates by the melanin pigments. For the erythaema measurement, two different wavelengths are used to measure the absorption capacity of the skin. One of these wavelengths corresponds with the spectral absorption peak of haemoglobin. The other wavelength has been chosen to avoid other colour influences (e.g., bilirubin). The results for both parameters are shown within 1 s as index numbers from 0–999. The same measuring probe is used to quantify both the skin redness (erythaema) and determine the degree of skin tanning (melanin)[16].

Fig. 1 shows the percentage changes determined after the 2nd, 4th, 6th, 8th, 10th, and 12th week and indicates that there was more decrease in erythaema exerted by the formulation as compared to base. However, by applying statistical ANOVA test, it was observed that the base produced insignificant ($p > 0.05$) effects on skin erythaema while formulation produced significant ($p \leqslant 0.05$) effects on skin erythaema with respect to time. Paired sample t-test showed that there were significant differences between the skin erythaema of the formulation as compared to base. This is also supported by the patch test at the beginning of study which indicated that both base and formulation did not irritate the skin. The decrease in skin erythaema can be due to anti inflammatory properties of fennel extract[17] containing flavonoids which include quercetin, ruinoside and kaempferol[18,19].

Skin sebum

Sebaceous glands present almost everywhere in body produce sebum which acts as lubricant and imparts waterproofing properties to the stratum corneum. Excessive sebum secretion leads to undesirable pathophysiological state called acne[20].

Skin sebum content was determined at 2-week intervals on the 2nd, 4th, 6th, 8th, 10th, and 12th

Table 2 Values of skin sebum at different time intervals after application of base (B) and formulation (F).

Volunteer #	0 h		Week 2		Week 4		Week 6		Week 8		Week 10		Week 12	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	45	51	49	49	55	58	49	54	51	47	53	43	38	39
2	75	84	73	80	81	74	75	68	88	73	89	64	77	57
3	82	93	88	89	92	89	86	85	99	93	96	77	87	74
4	78	86	65	94	73	83	77	81	83	72	86	79	93	73
5	66	75	63	71	69	69	70	77	72	65	74	62	79	59
6	79	84	84	98	89	96	92	91	95	103	99	87	101	79
7	112	109	116	103	108	111	115	101	122	86	112	88	119	84
8	126	118	132	112	137	108	141	106	149	87	155	85	161	82
9	73	81	90	96	86	74	92	71	75	98	71	93	84	91
10	129	122	133	117	142	109	138	106	135	83	144	98	155	93
11	117	126	122	106	116	105	127	93	134	87	121	104	145	95
Average	89	94	92	92	95	89	97	85	100	81	100	80	104	75
±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
SD	27	23	29	20	28	18	30	17	31	16	30	18	38	17

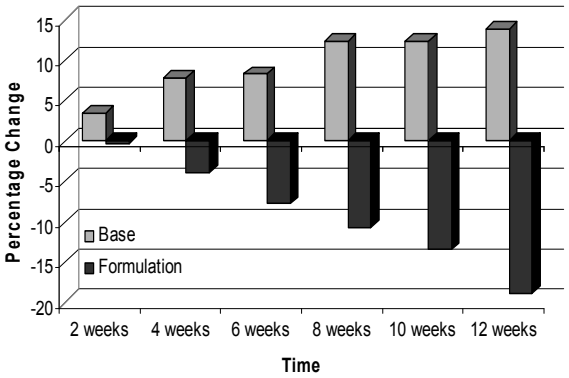

Fig. 2 Percentage of changes in skin sebum content after application of base and formulation.

week by Sebumeter MPA 5 and the values have been presented in Table 2 while percentage of changes are represented in Fig. 2. The measurement principle of Sebumeter MPA 5 is the photometric method. The sebumeter-cassette contains a mat synthetic tape. For each measurement the tape has to be transported forward by a trigger at the side of the cassette so that a new measuring section is exposed. For the determination of the sebum, the measuring head of the cassette is inserted into the aperture of the device, where a photocell measures the transparency. The light transmission represents the sebum content on the surface of the measuring area. A microprocessor calculates the result, which is shown on the display in values from 0–350[16].

In this study, the base increased the skin sebum content which can be due to the oily nature of the base having paraffin oil in it, but in the case of formulation there was a decline observed in skin sebum content throughout the study period. By applying ANOVA test, it was found that the base increased sebum insignificantly ($p > 0.05$) while the formulation had a significant ($p \leqslant 0.05$) decrease with respect to time. Paired sample t-test showed that the formulation produced significant effects in comparison with base.

Decrease in sebum content shown by the formulation can be possibly due to unsaturated fatty acids present in fennel which include oleic acid, linolenic acid, and linoleic acid[21]. Different studies have depicted that linolenic acid, linoleic, and oleic acids reduce sebum levels[14,22]. Topical application of linoleic acid has shown to inhibit sebum production due to selective inhibition of 5α-reductase, an enzyme found in sebaceous glands responsible for sebum production[23].

Skin melanin

Skin melanin was determined at 2-week intervals on the 2nd, 4th, 6th, 8th, 10th, and 12th week by Mexameter MPA 5 and the values are shown in Table 3 while the percentage changes are represented in Fig. 3.

In this study, the base increased the skin melanin values but in the case of formulation there was a regular decrease in skin melanin content throughout the study period. By applying ANOVA test, it was found that base increased melanin insignificantly ($p > 0.05$) while the formulation decreased skin melanin significantly ($p \leqslant 0.05$) with respect to time. By applying paired sample t-test, it was found that formulation produced significant ($p \leqslant 0.05$) effects with

Table 3 Values of skin melanin at different time intervals after application of base (B) and formulation (F).

Volunteer #	0 h		Week 2		Week 4		Week 6		Week 8		Week 10		Week 12	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1	332	334	343	328	340	317	339	312	356	306	364	292	342	287
2	265	254	270	243	276	236	278	225	266	217	258	211	264	207
3	401	408	404	398	413	401	408	405	412	407	416	404	421	398
4	433	440	442	434	428	428	425	448	422	439	413	444	440	437
5	352	361	344	365	340	368	360	361	358	364	362	360	368	356
6	377	376	380	385	384	391	372	386	368	376	375	387	387	380
7	341	345	352	354	350	346	354	340	344	325	336	333	347	338
8	323	347	328	340	333	338	337	329	345	322	351	320	346	312
9	429	454	446	457	449	451	441	443	435	435	426	426	442	419
10	397	394	405	399	411	402	416	398	419	393	422	387	428	390
11	372	486	380	397	375	386	361	380	384	379	388	370	381	366
Average	366	382	372	373	373	369	372	366	374	360	374	358	379	354
±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
SD	50	65	52	58	51	59	47	64	49	65	47	66	54	66

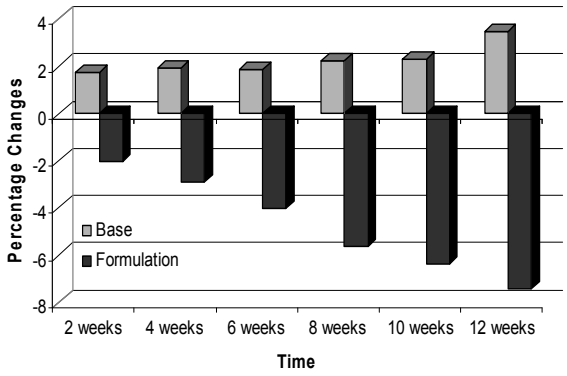

Fig. 3 Percentage of changes in skin melanin content after application of base and formulation.

respect to base.

The decrease in skin melanin can be attributed to the flavonoids and linoleic acid present in fennel extract. Linoleic acid is an unsaturated fatty acid and a major component of biological cell membranes. It is well known for having whitening effect on hyper pigmented skin[24]. Linoleic acid has been shown to speed up the degradation of tyrosinase which is involved in melanin synthesis and decrease tyrosinase levels. This ultimately leads to decreased melanin synthesis. UV-induced hyper-pigmentation of the skin has also been shown to decrease on treatment with formulations containing linoleic acid[25]. This implies that fennel extract cream containing linoleic acid exerts skin whitening effects.

CONCLUSIONS

We can conclude from this study that fennel (*F. vulgare)* exerts skin whitening effects as it significantly decreased skin melanin level. Decrease in skin sebum level suggests that the formulation can be helpful in conditions like acne. Decrease in skin erythaema showed that the formulation has anti-inflammatory effects when applied topically in the cream form. So it can be used as cost-effective topical skin-whitening treatment as well as sebum-reducing agent as the formulation exerts no harmful effects. Furthermore we suggest to investigate the effects of the formulation in unhealthy volunteers having acne and melasma to authenticate its use in diseased persons.

Acknowledgements: The authors wish to thank Higher Education Commission of Pakistan for providing financial support to conduct the study. The authors also acknowledge the moral support given by the Chairman and Dean of the Faculty of Pharmacy & Alternative Medicine, the Islamia University of Bahawalpur, Pakistan.

REFERENCES

1. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glower W (1999) Phenolic compounds and their role in oxidative processes in fruits. *Food Chem* **66**, 401–36.
2. Karakaya S, El SN, Taş AA (2001) Antioxidant activity of some foods containing phenolic compounds. *Int J Food Sci Nutr* **52**, 501–8.
3. Cieslik E, Greda A, Adamus W (2006) Contents of polyphenols in fruit and vegetables. *Food Chem* **94**, 135–42.
4. Majhenic L, Skerget M, Knez Z (2007) Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chem* **104**, 1258–68.

5. Farooq A, Ali M, Ijaz HA, Shahid M (2009) Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. *Flavour Frag J* **24**, 170–6.
6. Liu Q, Yao H (2007) Antioxidant activities of barley seeds extracts. *Food Chem* **102**, 732–7.
7. Zhong S, Yan WU, Soo MA, Zhao J, Wang K, Yang S, Jae HY, Xuejun ZHU (2006) Depigmentation of melanocytes by the treatment of extracts from traditional Chinese herbs: a cell culture assay. *Biol Pharmaceut Bull* **29**, 1947–51.
8. Oktay M, Gulçin I, Kufrevioglu I (2003) Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT Food Sci Tech* **36**, 263–71.
9. Javidnia K, Dastgheib L, Samani SM, Nasiri A (2003) Antihirsutism activity of fennel (fruits of *Foeniculum vulgare*) extract: A double-blind placebo controlled study. *Phytomedicine* **10**, 455–8.
10. Mimica DN, Kujund S, Sokovic M, Couladis M (2003) Essential Oil composition and antifungal activity of *Foeniculum vulgare* Mill. obtained by different distillation conditions. *Phytother Res* **17**, 368–71.
11. Özcan MM, Chalchat J-C, Arslan D, Ateş A, Ünver A (2006) Comparative essential oil composition and antifungal effect of bitter fennel (*Foeniculum vulgare* ssp. *piperitum*) fruit oils obtained during different vegetation. *J Med Food* **9**, 552–61.
12. Rasul A, Akhtar N, Khan BA, Mahmood T, Zaman SU, Khan HMS (2012) Formulation development of a cream containing fennel extract: in vivo evaluation for anti-aging effects. *Pharmazie* **67**, 54–8.
13. Hachem JP, Paepe KD, Vanpee E (2002) The effect of two moisturisers on skin barrier damage in allergic contact dermatitis. *Eur J Dermatol* **12**, 136–8.
14. Zaman SU, Akhtar N, Khan BA, Mahmood T, Rasul A, Mahmood A, Muhammad Aamir MN, Ali A (2012) Development of a sebum control cream from a local desert plant *Capparis deciduas*. *J Medicinal Plant Res* **6**, 744–8.
15. Bosnjak S (2001) The Declaration of Helsinki—the cornerstone of research ethics. *Arch Oncol* **9**, 179–84.
16. Courage W, Khazaka G (2004) Information and operating instructions for the multi probe adapter MPA and its probes. Available at http://www.courage-kazaka.de.
17. Choi EM, Hwang JK (2004) Anti-inflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. *Fitoterapia* **75**, 557–65.
18. Parejo I, Viladomat F, Bastida J, Schmeda HG, Burillo J, Codina C (2004) Bioguided isolation and identification of the non-volatile antioxidant compounds from fennel (*F. vulgare* Mill.) waste. *J Agr Food Chem* **52**, 1890–7.
19. Cherng JM, Chiang W, Chiang LC (2008) Immunomodulatory activities of common vegetables and spices of Umbelliferae and its related coumarins and flavonoids. *Food Chem* **106**, 944–50.
20. Giacomoni PU, Mammone T, Teri M (2009) Gender-linked differences in human skin. *J Dermatol Sci* **55**, 144–9.
21. He W, Huang B (2011) A review of chemistry and bioactivities of a medicinal spice: *Foeniculum vulgare*. *J Medicinal Plant Res* **5**, 3595–600.
22. Akhtar N, Khan BA, Mahmood T, Parveen R, Qayum M, Anwar M, Zaman SU, Farooq M (2010) Formulation and evaluation of antisebum secretion effects of sea buckthorn w/o emulsion. *J Pharm Bioall Sci* **2**, 13–7.
23. Mahmood T, Akhtar N, Khan BA, Khan HMS, Saeed T (2010) Outcomes of 3% green tea emulsion on skin sebum production in male volunteers. *Bosnian J Basic Med* **10**, 260–4.
24. Shigeta Y, Imanaka H, Ando H, Ryu A, Oku N, Baba N, Makino T (2004) Skin whitening effect of linoleic acid is enhanced by liposomal formulations. *Biol Pharmaceut Bull* **27**, 591–4.
25. Ando H, Mary S, Ichihashi M (2010) Quasi-drugs developed in Japan for the prevention or treatment of hyperpigmentary disorders. *Int J Mol Sci* **11**, 2566–75.