OUTCOMES OF 3% GREEN TEA EMULSION ON SKIN SEBUM PRODUCTION IN MALE VOLUNTEERS

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Abstract

This study was aimed to depict potential effects of stable formulation (water in oil emulsion), containing 3% green tea (*Camellia sinensis* L) extract on skin sebum production in healthy human volunteers.

For this purpose formulation was designed using 3 % ethanolic green tea extract and Abil®EM90 was used as an emulsifier. Formulation was applied to the cheeks of healthy human volunteers (n=10) for a period of 8 weeks. Measurements for skin sebum production were considered using Sebumeter MPA 5. Results were compiled and any effect produced by the formulation was justified statistically. It was observable that statistically significant ($p < 0.5$ %) results were found for skin sebum production after long term application of the formulation. 3% formulation of green tea extract was ideal in all aspects and can be experienced in skin disorders like acne to further investigate its effects in unhealthy volunteers.

KEY WORDS: *Camellia sinensis* L, ANOVA, skin sebum, Abil®EM90, formulation.

Introduction

Skin is considered excellent model for non-invasive topical delivery. Topical emulsions have widespread acceptability by the user and have been used as dermatological formulations since long time (1). Water in oil type emulsions are frequently used because of their ease of application, restriction of water evaporation from skin surface and by their emollient action (2). More than 3000 years ago in Chinese history, emperor Sin-Non described that a daily cup of tea could be handy to dissolve number of poisons in our body. It was strongly believed in ancient culture that tea can prevent and treat many diseases. But scientifically these beneficial effects are described more recently (3). Tea (Camellia *sinensis* L) of Theaceae has many kinds but three well known types are: green (un-fermented), oolong (semi-fermented), and black (fully-fermented). By inactivating the enzymes in the fresh leaves green tea is made either by firing or by steaming, thus to prevent the enzymatic oxidation of catechins (4). Catechins (polyphenols) are main active constituents found in tea (5). There are several polyphenolic catechins in green tea, viz. (–) epicatechin (EC), (–) epicatechin3gallate (ECG), (–) epigallocatechin(EGC), (–) epigallocatechin-3-gallate (EGCG), (+)catechin, and (+) gallocatechin (GC) Figure 1. EGCG is the most found catechin in green tea (65%) of the total catechin contents (6). Tea preparations have shown to trapping activity against various reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radical, superoxide radical, nitric oxide, peroxynitrite and nitrogen dioxide and were helpful in reducing damage to proteins, lipid membranes and nucleic acid in cell-free systems (7). Furthermore epidemiological studies suggest that tea is effective against variety of cancers and well known chemo preventive agent. Several other epidemiological studies suggest green tea effective in oral diseases, bone disorders, neurodegenerative and cardiovascular disorders (8). The problem with green tea polyphenols was their strong affinities for many proteins like milk, casein and their interference with digestive enzymes that results in reduced lipid, starch and proteins digestibility. They have shown to interfere with zinc, iron and sodium as well (9). Moreover catechins are subject to extensive biotransformation including methylation, glucuronidation, sulfation and ring fission metabolism. The potential health effects of catechins depend on the amount consumed and on their bioavailability. The plasma concentrations of EGCG were much lower when given orally (7). Aim of this study was to test this formulation in healthy human volunteers for its effects on skin sebum production as many of the active constituents in green tea are prompted to contain anti-sebum effects declared by a study that green tea decreases hormonal activity because tea galltes and α- linoleic acid are selective inhibitors of 5 α-reductase (10). Furthermore green tea reduces inflammation due to lipids present in green tea such as Linoleic acid and α- linoleic acid (8) which reduces microcomedone size in micrcomedonal acne (11). It has antimicrobial activity (12), *P. acnes* was most sensitive with green tea flavor compounds (13). Green tea is also powerful antioxidant (14). Therefore keeping in mind all above points of view, a desired dermatological formulation was of esteemed value using green tea extract.

Material and Methods

Detailed procedure is previously reported in (20) and briefly summarized here. *Camellia sinensis* leaves extract was prepared by maceration. For this purpose 200 grams powdered *Camellia sinensis* leaves were drenched with analytical grade ethanol for 21 days. Filtered extract was evaporated at

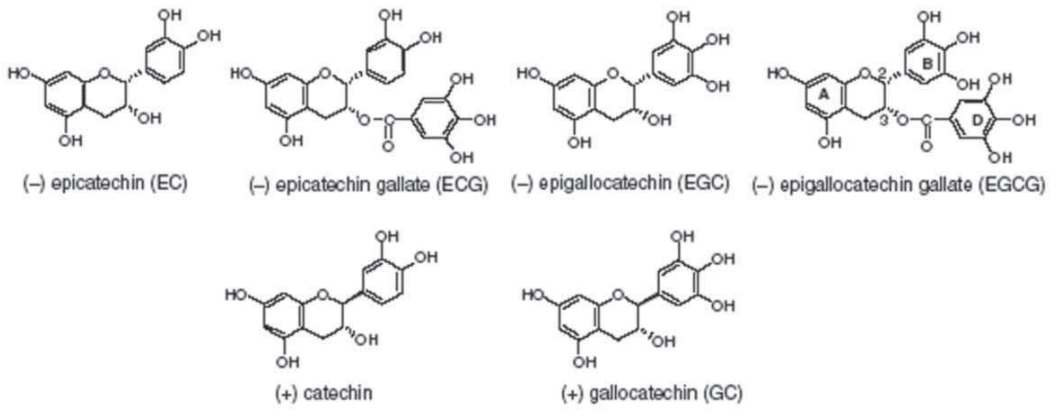

FIGURE 1. Structures of the major catechins (polyphenols) present in green tea

40°C under vacuum, using a rotary evaporator. The blackish green colored extract was stored at 8°C. For a formulation paraffin oil (16 %) and surfactant Abil®EM90 (5 %) was heated up to 75°C±1°C. At the same time, aqueous phase consisting of water (quantity sufficient to make 100 %) was heated to 75°C±1°C and then the green tea extract (3 %) was added in this aqueous phase. After that, aqueous phase was added to the oil phase drop by drop and stirred with homogenizer to obtain a formulation with dependable consistency. Mean while few drops of lemon oil were added during this stirring time to give good fragrance to the formulation.

Study design

For application of formulation, 10 volunteers were chosen whose ages were in between 25 and 40 years. Only male volunteers were included in this work. Volunteers were examined for any serious skin disease or damage especially on cheeks and forearms. Each volunteer was provided with a volunteer protocol before the study. This protocol stating each volunteer signed the terms and conditions of the testing individually. Volunteers were not informed about the contents of the formulations. Skin tests were performed at 25°C and 40% relative humidity conditions. Before application of formulation a patch test was performed on forearms of the volunteers for 48 hours to check any irritation in the formulation. Each volunteer on the second day was provided with formulation and volunteers were instructed properly about the application of formulation. Each individual was instructed to come for measurements of readings for skin sebum production on week 1, 2, 3, 4, 6, and 8.

Mathematical and Statistical Analysis

The percentage changes for the individual values of different parameters, taken every week, of volunteers were calculated by the following formula;

$$\text{Percentage Change} = ((A - B) / B)*100$$

Here;

A = Individual value of any parameter (from 1st to 8th week)

B = Zero hour value of that parameter

The measured values obtained for skin sebum production were analyzed using SPSS 12.0 on the PC computer (ANOVA for variation between different time intervals and level of significance was 0,5 %).

Results and Discussion

In-vitro evaluation of creams

Stability of the formulation was evaluated using different conditions of storage i.e. 8°C, 25°C and 40°C + 75% RH (relative humidity). In this study no liquefaction and phase separation were observed in the formulation samples throughout the study period of 28 days even at elevated temperatures. Abil®EM90 is a lipophilic surfactant (15) and it has been found that lipophilic surfactants are more stable at elevated temperatures (16). Furthermore pH of the formulation was 5.67, considered as normal skin pH range i.e. 4 to 6,5 (17). Detailed description is previously reported in (20).

In-vivo characterization of the formulation for skin anti-sebum effects

Sebum is produced by halocrine sebaceous glands connected to the hair follicles. Sebaceous glands are found everywhere in the body except in palm and soles. Sebum acts as lubricant and imparts waterproofing properties to the stratum corneum. Any excessive sebum secretion is associated with pore enlargement leading to undesireable pathophysiological condition called acne (18). Topical application of α- linoleic acid and EGCG ((-)-eppigallocatechin-3-gallate) in animal model have shown to inhibit sebum production. This

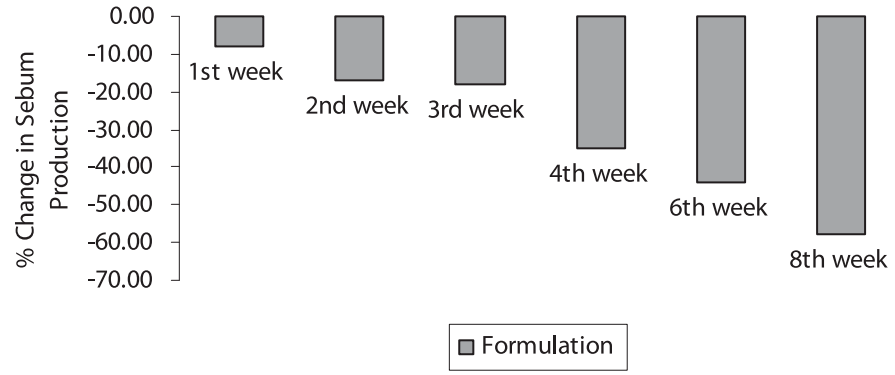

FIGURE 2. Percentage of change in skin sebum production after application of the formulation

activity was due to selective inhibition of 5α-reductase (3), an enzyme found in sebaceous glands (19). There are two isozymes for 5α-reductase, type 1 and type 2. As α- linoleic acid inhibits both type 1 and type 2 while EGCG inhibits type 1 at concentration of 30 mmol/l (3). In this study effects of the formulation on skin sebum production were noted in volunteers and results for average percent changes in skin sebum production are presented in Figure. 2.

It was found that formulation decreased the sebum contents throughout the study period of 60 days. When justified statistically results of ANOVA were significant ($p < 0.5$) for the formulation with respect to time. Up to our knowledge this is first study of its kind in which 3% green tea extract was tested for anti-sebum secretion effects in male healthy volunteers. Further propagation of this study in unhealthy states would be more imperative to state the actual potential of 3% green tea emulsion. Further study could be based on the study design by Sharquie et al would be sound enough to test this emulsion in unhealthy states like acne vulgaris. In this study they have compared 2% tea lotion with 5% zinc sulfate solution in the treatment of acne. Between the two groups studied in this study, group treated with 2% tea lotion was found more effective in decreasing number of inflammatory lesions than 5% zinc sulfate solution. It was concluded from this study that 2% tea lotion was more superior in its effects and could be superior choice than 5% zinc sulfate solution in treatment of acne vulgaris (21). We also suggest that cheaper 3% green tea emulsion without any added substances could be tested in conditions like acne vulgaris against any standard treatment to compare its results in unhealthy states.

Conclusion

Above mentioned results for formulation seems to promote the concept about green tea multi factorial benefits against sebum production i.e. tea galltes and α- linoleic acid are selective inhibitors of 5 α-reductase (10), α- linoleic acid reduces microcomedone size in micrcomedonal acne (11). Further propagation of this study in unhealthy volunteers might be foundation to speculate about the functionality of green tea extracts delivered topically in opposition to sebum production. Hence we suggest that cheaper 3% green tea emulsion should be tested in conditions like acne vulgaris against any standard treatment to compare its results in unhealthy states.

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Conflict-of-interest policy

Authors do not have any commercial affiliations, or potential conflicts of interest associated with this work submitted for publication.

Ethical Standards

In this study, the clinical research using human subjects was conducted in accordance with the ethical considerations for human subjects approved by Board of Advance Studies and Research. Healthy human volunteers (male) were selected whose ages were in between 25 and 40 years. Prior to the tests, the volunteers were examined by a cosmetic expert for any serious skin disease or damage especially on cheeks and forearms. Before the study, every volunteer was provided with a volunteer protocol. This protocol stating the terms and conditions of the testing were signed by every volunteer individually. Volunteers were not informed about the contents of formulations. All the skin tests were done at 25°C and 40% relative humidity conditions. On the first day, patch test (i.e. skin sensitivity test) was performed on the forearms of each volunteer to determine any possible reactions to the topical emulsion.

Abbreviations

ROS - Reactive Oxygen Species
EGCG - epigallocatechin-3-gallate

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