Evaluation of various skin mechanical properties after application of cosmetic emulgel loaded with *Piper nigrum* seed extract

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ABSTRACT

The aim of this study was to evaluate changes in skin erythema, skin melanin, skin hydration, skin elasticity, skin sebum and skin number of pores during three months application of cosmetic emulgel loaded with *Piper nigrum* seed extract. Base without plant extract and 4% plant extract loaded emulgel was prepared. The base and plant extract loaded formulation were applied on cheeks of healthy volunteers (n=13). Hydration level of skin was statistically significantly ($p<0.05$) increased by plant extract loaded formulation. The formulation demonstrated a statistically significant ($p<0.05$) decrease in skin melanin content while the base had insignificant ($p > 0.05$) effects. The formulation greatly decreased skin erythema level ($p<0.05$). Regarding sensory evaluation, both emulgel were aesthetics. The topical, non-invasive

application of *Piper nigrum* seeds extract loaded emulgel shown a remarkable decrease in number of pores on human skin. This study will bolster interest in research and conviction in the use of natural remedies.

INTRODUCTION

Today, diseases have effectively managed by giving medications to people's bodies through a variety of routes available (1, 2). Topical drug delivery refers to the applying of a medication product to the skin for the relief of cutaneous illnesses (3). The formulas and textures of dermatological skin care treatments range from liquid to powder, although semi-solid products are the most often utilised. Within this major category of semisolid preparations, the use of transparent gels has increased in both pharmaceutical and cosmetic products. To create gels, a more contemporary kind of dosage forms, enormous amounts of hydrophilic or hydroalcoholic liquid are encased in a structure made up of colloidal solid particles. When gels and emulsions are used together, a dosage form called Emulgel is created. Lipophilic pharmaceuticals are encapsulated using the direct O/W method, whereas hydrophilic medications are encapsulated using reverse W/O system (4). Emulsions may be readily removed anytime needed, have a high capacity to enter the skin, and have a certain level of elegance. Emulgels for dermatological usage are thixotropic, greaseless, readily spreadable, easily removed, emollient, non-staining water-soluble, prolonged shelf life, bio-friendly translucent, and have a beautiful look (5).

P. nigrum is a member of the Piperaceae family. Piperine, alkaloids, amides, safrol, terpenes, and dietary fibres are all components of *P. nigrum*. P. nigrum has antioxidant activity and can serve as an easily obtainable source of organic radical scavenging activity in the food and cosmetic industries because to its high phenolic content. *P. nigrum* having piperine have a wide range of pharmacological effects, including analgesic, antimicrobial, antioxidant, anti-diarrheal, anticonvulsant, and antitumor properties. They also have antidepressant anti-depressant, anti-hypertensive, and antiplatelet properties. The aim of this study was to test the emulgel formulation utilizing non-invasive bioengineering techniques on healthy human volunteers for three months to evaluate the different skin mechanical properties.

MATERIALS AND METHOD

The material and method for the preparation of cosmetic emulgel *P. nigrum* and its characterization (in-vitro) already described and published (https://doi.org/10.3390/molecules27185990)

PROTOCOL OF STUDY

Randomized, single-blinded trial was devised for a 12-week non-invasive study of emulgel formulations and base on human skin. The Institutional Ethical Review Committee (No. 45/S-2017/PREC) and Research Board (No. 496/AS&RB) both approved the study. All skin parameters were measured by the international standards of the Helsinki Declaration, International Ethical Guidelines for Biomedical Research Involving Human Subjects by The Council for International Organizations of Medical Sciences (CIOMS) (6), at the Cosmetic Lab. Thirteen (n=13) people in total, ranging in age from 20 to 40, were selected (male, healthy, insensitive, and non-smokers). All participants were given information regarding the study's purpose, potential risks, and methods before the study. They also given sign a written permission form as confirmation that they understood the study's terms and conditions. All participants were evaluated by a dermatologist for any type of disorder, especially in the study's skin regions. The participants were instructed to adhere to their regular diet and refrain from taking skin treatment to rule out the possibility of potentiation by the research product. A controlled atmosphere with a temperature of 25°C and a relative humidity of 40% was used for all of the skin testing. Before each evaluation of skin parameters, volunteers were asked to spend 15 minutes sitting normally in the cosmetic lab so they could become acclimated to the humidity and temperature levels inside. A placebo and an active formulation, labeled "Left" and "Right" to indicate which cheeks they should be placed on, were provided to each participant. Asked to apply each of the formulas to their cheeks twice daily. Measurements were taken at the beginning of the experiment, as well as during weeks two, four, six, eight, ten, and twelve. In vivo studies involving human volunteers started from 20th February 2020 till 20th May, 2020.

RESULT AND DISCUSSION

PATCH (BURCHARD) TEST

Data of the patch test demonstrate that neither the tested formulations nor the placebo caused any volunteers to experience any sort of itchiness or allergy, as shown in Figures **1-A**.

SKIN'S ERYTHEMA INDEXES

The skin's erythema index was assessed at regular intervals beginning at the start of the trial and continuing through weeks 2, 4, 6, 8, and 12 of the study. Variations in erythema index caused by both the formulation and the placebo were noted. The mean of each reading was calculated after being measured in triplicate. Figure **1-B** depicts the percentage increase or decrease in skin erythema index.

SKIN'S MELANIN INDEXES

Skin's melanin score was assessed at regular intervals commencement at the beginning of the trial and continuing through weeks 2, 4, 6, 8, and 12; changes in melanin index caused by the placebo and the investigated formulations were noted. The mean of each reading was calculated after being measured in triplicate. Figure **1-C** displays the percentage change in the skin melanin index

SKIN'S HYDRATION INDEXES

At regular intervals commencing at baseline, the skin's hydration level was assessed at weeks 2, 4, 6, 8, and 12 of the trial, and changes in hydration level caused by the investigation's formulations and the placebo were noted. The mean of each reading was calculated after being measured in triplicate. Figure **1-D** displays the percentage change in the skin moisture level index.

EVALUATION OF SKIN ELASTICITY INDEXES

The elasticity index of skin was measured at regular intervals at zero time and then at 2nd, 4th, 6th, 8th and 12 weeks of the study and change in elasticity index by placebo and the studied formulations was recorded. All the readings were measured as triplicate and mean was taken. Percentage change in skin elasticity index is represented graphically in **Figure 1-E.**

SKIN'S SEBUM INDEXES

The skin's sebum score was examined at regular intervals at the beginning of the trial, at weeks two, four, six, eight, and twelve, and a change in skin sebum index due to the placebo and the investigated formulations was noted. **Figure 1-F** shows a graphical illustration of the percentage change in sebum score.

EVALUATION OF FACIAL PORES

The average percentage variation in facial skin pores (both large and small pores) was evaluated. after the administration of each formulation and its corresponding placebo at the appropriate time intervals of the second, fourth, sixth, eighth, tenth, and twelfth week throughout the three-month study period. To reduce the possibility of error, the values are measured in triplets. Images from Visoface were used to display the pore size analyses.

The participants were tested for any sort of hypersensitivity or erythema that might occur as a result of applied formulations. This might jeopardize the safety of the individuals who are being prescribed these drugs. As a result, skin irritation tests must be conducted for newly produced formulation (7). The patch test was once used to assess the security of a composition. In patch testing, only a single application is utilized to verify the formulation's acute irritating response. (8). During the patch test, neither the formulations nor the control showed any skin irritation, and the skin erythema content reduced after 48 hours, as shown in the graph **Figure 1-A**. The antioxidant activity and polyphenolic content of the extracts included in the emulgels may be responsible for the decrease in erythema content (9). This demonstrates that all emulgels may be safely applied to human skin for in vivo testing.

Erythema and inflammation in the skin compromise its protective system, leading to further negative consequences (10). But the most important thing is to find natural products that can minimize erythema and prevent skin damage. The results demonstrated that, the formulation consistently reduced the erythema over the course of the 12-week research. According to the findings, the formulation reduced the erythema index by up to 12 percent when compared to the baseline. Significant change ($p<0.05$) in erythema by formulation and insignificant change ($p>0.05$) by placebo were observed. with the respective placebo, according to statistical analysis using ANOVA with a 5% level of significance. Non-enzymic antioxidants, such as, phenol, piperine, campesterol, total carotenoids, isoelemicin, oleic acid, squalene, caryophyllene,

flavonoids, gamma-seterol, are important for biological systems in reducing reactive oxygen species are present in *P.nigrum* seed extract. Carotenoids have been shown to improve skin UV protection and reduce susceptibility to UV-induced erythema when consumed in large amounts. The inclusion of antioxidants such as carotenes, sigmasterol, phenol, oxaspiro, oleic acid, and *n*-hexadecanoic acid may be responsible for the formulation's reduction in skin erythema.

Skin coloration is caused by the presence of melanin pigment in human skin. Bacteria, fungi, and plants all contain the melanin pigment. The enzyme tyrosinase is responsible for the formation of melanin (11). Tyrosinase over-activity causes an excess of melanin to be produced, resulting in hyperpigmentation of the skin, whereas underactivity causes vitiligo (skin depigmentation) and hair whitening. As a result, inhibiting the tyrosinase enzyme might result in less melanin synthesis. Natural and synthetic substances in topical preparations are frequently utilized in the treatment of hyperpigmentation. Ellagic acid, licorice, N-acetyl glucosamine, rucinol, green tea, azelaic acid, dioic acid (12).The control emulgel raised skin melanin content, while the test formulations lowered skin melanin content in this present study. Following the use of the formulation, the melanin index decreased dramatically from -4 percent (week 2) to -9.20 percent (week 12), as seen in **Figure 1-C**. The results of the statistical analysis using two-way ANOVA at a 5% significance level revealed that all of the formulations caused a significant change ($p<0.05$) in skin melanin content, but the impact was insignificance ($p>0.05$) in the control emulgel. The phenolics and flavonoids included in *p. nigrum* seed extract may be responsible for the decrease in skin melanin concentration. Flavonoids' tyrosinase inhibitory action might be attributed to chelating the active core of the tyrosinase enzyme, resulting in a synthesis of melanin contents (13). The presence of tocopherols, Phenol, oleic acid, and n- Hexadecanoic acid in the extract can also be linked to the skin depigmenting and antioxidant activities of *p. nigrum* seed extract.

The main selection criteria for skin care products also take into account how well the skin's various layers retain moisture. With advancing age and changes in the external environment, the tissues that retain moisture in skin are destroyed. The skin loses its suppleness and becomes dry and wrinkled when the water content is reduced to 10%. Due to their excellent moisture retention capacity and beneficial effects on skin nutrition, biological plant extracts are replacing synthetic moisturising actions (14). According to this study's analysis of the percent change in moisture

contents following application of each emulgel, skin moisture contents gradually increased after repeated application for 12 weeks. The percentage of the plant extract-containing emulgel increased noticeably from 18 percent (week 2) to 42.10 percent (week 12). The two-way ANOVA statistical analysis at a 5% level of significance revealed that the formulation considerably ($p<0.05$) raised skin hydration levels, whereas the placebo had an insignificance ($p>0.05$) effect. In addition, Profilaggrin is the main epidermal barrier responsible for maintaining the skin's level of moisture (15). Flavonoids found in *piper nigrum* seed extracts cause filaggrin to be upregulated, which leads to its differentiation and contributes to its moisturising ability. Additionally, during this investigation, the control emulgel increased the skin moisture index. The principal ingredient in both the formulation and the placebo, paraffin oil, may be to reason for this increase (16).

The continual generation of collagenase and increased likelihood of inflammatory responses all contribute to the skin's persistent exposure to UV light. Collagen, which is necessary to keep the skin elastic, is found in fibroblasts found in the dermis of human skin (17). Skin aging is distinguished by a loss of skin elasticity or a reduction in skin elasticity (18). In this study, the impact of emulgels containing antioxidant extracts has been evaluated on skin elasticity, and the percentage change is from 2% (week 2) to 24.60% (week 12), while the change in case of placebo is from -2.0 percent (week 2) to -12.20 percent (week 12). The results of the statistical analysis using a two-way ANOVA test with a 5% threshold of significance revealed that all test formulations significantly changed the skin's elasticity ($p<0.05$) but the control generated an insignificant change ($p>0.05$).The maintenance of the skin's healthy texture is intricately linked to the presence of carotenes in the skin (19).The use of carotenoids in cosmetic formulations is thought to contribute to skin regeneration and epidermis renewal while also advancing epidermal layer thickness. Numerous polyphenols, phenolic acids, and other antioxidants have demonstrated beneficial effects on human skin and have also been linked to increased skin flexibility. The inclusion of carotenoids, phenolic acids, and vital vitamins may explain how *P.nigrum* extract improves skin elasiticity. Additionally, phenolic acids and vitamin have been found in *P.nigrum* seed extract.

Sebum is a human bodily fluid that protects the skin against microorganisms and toxins while also maintaining its emollient properties (20). Sebum that is produced and added in excess causes oily skin, which can lead to seborrhea and acne (21). By providing anaerobic and lipid-rich

air for the growth of the acne-causing pseudomonas bacterium, excessive sebum levels in human skin were found to be implicated in the pathogenesis of acne (22). Due to the herb's antioxidant, anti-androgen, antibacterial, and anti-inflammatory properties, herbal treatment is frequently used to treat acne. In this study, the sebum level was decreased with an average percentage shift from -5 percent (week 2) to -21.20 percent (week 12) as shown in figure 17.A two-way ANOVA statistical analysis with a 5% threshold of significance ($p<0.05$) revealed that all test formulations significantly changed the amount of skin sebum whereas the control had an insignificant effect ($p>0.05$). The earlier literature is being strengthened by this drop in skin sebum levels. Due to the creation of dihydrotestosterone, the -1 reductase is entangled in the development of sebaceous glands (excessive sebum excretion). This -1 reductase enzyme is obstructed by several phytochemical components such polyphenols and sterols, which are also involved in lowering the sebum levels (18). Additionally, it is stated that plant extracts containing lipids and polysterols non-specifically block 5-reductase and regulate the synthesis of sebum (23). *P.nigrum* root extract has demonstrated the presence of phytosterols, steroids, and terpenoids, among other substances.

The most prevalent issue in cosmetology is the increase of large facial pores since it leads to very rough complexions. Cosmetologists are attempting to develop formulations that lessen the number of large facial pores.

CONCLUSION

These findings demonstrate the antioxidant and UV protection properties of emulgels containing *P. nigrum* seed extract (4%). In order to protect the skin from harmful UV sun rays, it can be utilized. The improved formulations achieved extremely high volunteer acceptability and caused no skin discomfort. On skin metrics including melanin, erythema, sebum, moisture, and elasticity, the improved formulations showed extremely good cosmeceutical results. The developed emulgel composition can therefore be used as a natural alternative to preparations containing synthetic ingredients for skin protection. Additionally, the emulgel formulation was created utilizing readily accessible, moderately priced components and basic equipment, all of which will help make this formulation's creation in the pharmaceutical business more affordable in the future.

REFERENCES

1. Verma P, Thakur A, Deshmukh K, Jha A, Verma S. Routes of drug administration. International Journal of Pharmaceutical Studies and Research. 2010;1(1):54-9.
2. Leaf-nosed bat. Encyclopædia Britannica: Encyclopædia Britannica Online; 2009.
3. Leppert W, Malec–Milewska M, Zajaczkowska R, Wordliczek J. Transdermal and topical drug administration in the treatment of pain. Molecules. 2018;23(3):681.
4. Khullar R, Saini S, Seth N, Rana A. Emulgels: a surrogate approach for topically used hydrophobic drugs. Int J Pharm Bio Sci. 2011;1(3):117-28.
5. Jain A, Gautam SP, Gupta Y, Khambete H, Jain S. Development and characterization of ketoconazole emulgel for topical drug delivery. Der pharmacia sinica. 2010;1(3):221-31.
6. van Delden JJ, van der Graaf R. Revised CIOMS international ethical guidelines for health-related research involving humans. Jama. 2017;317(2):135-6.
7. Vinardell M, Mitjans M. Alternative methods for eye and skin irritation tests: an overview. Journal of pharmaceutical sciences. 2008;97(1):46-59.
8. Gaspar L, Camargo Jr F, Gianeti M, Campos PM. Evaluation of dermatological effects of cosmetic formulations containing Saccharomyces cerevisiae extract and vitamins. Food and chemical toxicology. 2008;46(11):3493-500.
9. Arshad AI, Khan S, Akhtar N, Mahmood A, Sarfraz RM. In vivo evaluation of skin irritation potential, melasma and sebum content following long term application of skin care cream in healthy adults, using non-invasive biometrological techniques. Acta Pol Pharm. 2016;73:219-27.
10. Bhat BB, Kamath PP, Chatterjee S, Bhattacherjee R, Nayak UY. Recent Updates on Nanocosmeceutical Skin Care and Anti-Aging Products. Current Pharmaceutical Design. 2022.
11. Hariri R, Saeedi M, Akbarzadeh T. Naturally occurring and synthetic peptides: Efficient tyrosinase inhibitors. Journal of Peptide Science. 2021;27(7):e3329.
12. Konda S, Geria AN, Halder RM, editors. New horizons in treating disorders of hyperpigmentation in skin of color. Seminars in cutaneous medicine and surgery; 2012: WB Saunders.
13. Saewan N, Koysomboon S, Chantrapromma K. Anti-tyrosinase and anti-cancer activities of flavonoids from Blumea balsamifera DC. Journal of Medicinal Plants Research. 2011;5(6):1018-25.
14. Arshad W, Khan HMS, Akhtar N, Mohammad IS. Polymeric emulgel carrying Cinnamomum tamala extract: promising delivery system for potential topical applications. Brazilian Journal of Pharmaceutical Sciences. 2020;56.
15. Ovaere P, Lippens S, Vandenabeele P, Declercq W. The emerging roles of serine protease cascades in the epidermis. Trends in biochemical sciences. 2009;34(9):453-63.
16. Rawlings A, Lombard K. A review on the extensive skin benefits of mineral oil. International journal of cosmetic science. 2012;34(6):511-8.
17. Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ, et al. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. The American journal of pathology. 2006;168(6):1861-8.
18. Dobrev H. Clinical and instrumental study of the efficacy of a new sebum control cream. Journal of cosmetic dermatology. 2007;6(2):113-8.
19. Camejo D, Martí MaC, Roman P, Ortiz A, Jimenez A. Antioxidant system and protein pattern in peach fruits at two maturation stages. Journal of agricultural and food chemistry. 2010;58(20):11140-7.
20. Zaman S, Akhtar N. Effect of turmeric (Curcuma longa Zingiberaceae) extract cream on human skin sebum secretion. Tropical Journal of Pharmaceutical Research. 2013;12(5):665-9.

21. Laneri S, Dini I, Tito A, Di Lorenzo R, Bimonte M, Tortora A, et al. Plant cell culture extract of Cirsium eriophorum with skin pore refiner activity by modulating sebum production and inflammatory response. Phytotherapy Research. 2021;35(1):530-40.
22. Jović A, Marinović B, Kostović K, Čeović R, Basta-Juzbašić A, Bukvić Mokos Z. The impact of psychological stress on acne. Acta Dermatovenerologica Croatica. 2017;25(2):133-.
23. Zhang L-C, Hu J-H, Li L, Gao L-H, Zhu Q-G, Li Z, et al. In Vivo and in Vitro Evaluation of Essential Oils from Ligusticum chuanxiong H ORT on the Transdermal Delivery of Flurbiprofen in Rabbits. Biological and Pharmaceutical Bulletin. 2006;29(6):1217-22.

LIST OF FIGURES

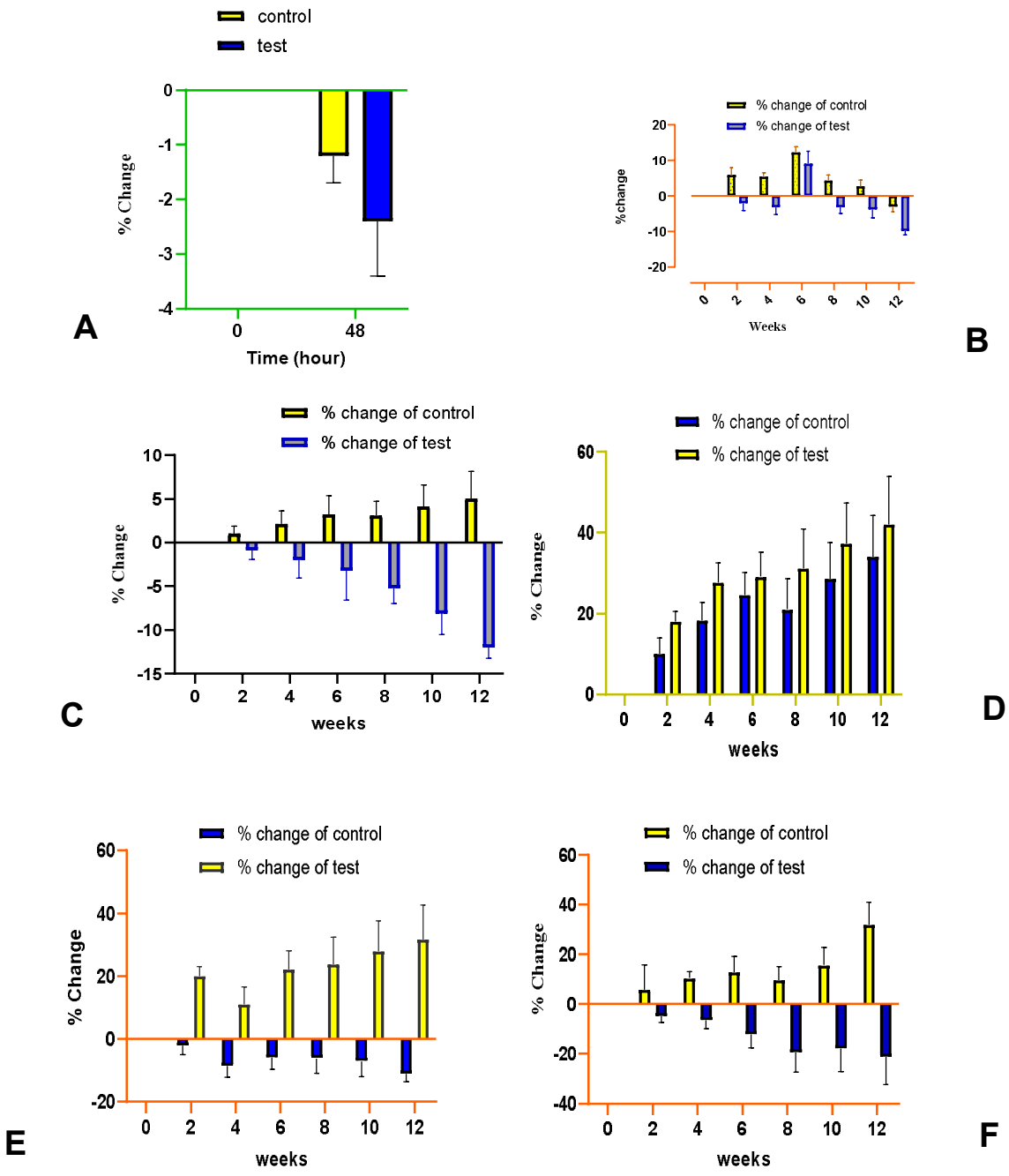

Figure 1. (A) Erythema variation for primary irritation by both placebo and formulation. **(B)**. Erythema index after treatment of 12 weeks. **(C)** Placebo and formulation on melanin after 12 weeks treatment. **(D)** Placebo and formulation on skin's hydration after 12 weeks treatment. **(E)** Placebo and formulation on elasticity index after 12 weeks treatment. **(F)** Placebo and formulation on sebum index after 12 weeks treatment