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CALENDULA EXTRACT: EFFECTS ON MECHANICAL PARAMETERS OF HUMAN SKIN

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Abstract: The aim of this study was to evaluate the effects of newly formulated topical cream of *Calendula officinalis* extract on the mechanical parameters of the skin by using the cutometer. The Cutometer® 580 MPA is a device that is designed to measure the mechanical properties of the skin in response to the application of negative pressure. This non-invasive method can be useful for objective and quantitative investigation of age related changes in skin, skin elasticity, skin fatigue, skin hydration, and evaluation of the effects of cosmetic and antiaging topical products. Two creams (*base* and *formulation*) were prepared for the study. Both the creams were applied to the cheeks of 21 healthy human volunteers for a period of eight weeks. Every individual was asked to come on week 1, 2, 3, 4, 5, 6, 7, and 8 and measurements were taken by using Cutometer® MPA 580 every week. Different mechanical parameters of the skin measured by the cutometer were; R0, R1, R2, R5, R6, R7, and R8. These were then evaluated statistically to measure the effects produced by these creams. Using ANOVA, and t-test it was found that R0, and R6 were significant ($p < 0.05$) whereas R1, R2, R5, R7, R8 were insignificant ($p > 0.05$). The instrumental measurements produced by *formulation* reflected significant improvements in hydration and firmness of skin.

Keywords: cutometer, skin elasticity, skin hydration, skin fatigue, antiaging

The proper functioning and the appearance of the skin are maintained by an important balance between the water content of the stratum corneum and skin surface lipids. This balance is disrupted by exposure to external factors like: air humidity, UV radiation, temperature and hormones (1). The dermis is largely made up of interwoven fibers, principally of collagen, packed in bundles. Those in the papillary dermis are finer than those in the deeper reticular dermis. When the skin is stretched, collagen, with its high tensile strength, prevents tearing, and elastic fibers, intermingled with the collagen, later return to its unstretched state (2).

As the process of aging occurs, a lot of changes are observed in our skin. More wrinkles and pigmentation are seen, there is less moisture and lipids and the skin also becomes sagged because of the loss of its elasticity. The evaluation of the skin elasticity is very important, because it is not as visible as other signs of aging such as wrinkles (3). It has been demonstrated that with the process of aging, the elastic fibers of the papillary dermis progressively disappear and lose their branched appearance (4).

Skin mechanical parameters are very sensitive to epidermal hydration. The mechanical properties of skin are greatly influenced by the epidermal hydration produced by the moisturizers. The non-invasive skin elasticity measurements are appropriate for an objective and quantitative evaluation of the complex effect of different dermatological and cosmetic products on epidermal mechanics and water content (5). Skin suction experiments are usually conducted to evaluate the mechanical properties of the skin. In such experiments, the skin is elevated into a circular aperture by the use of low pressure. While performing the experiment, the skin elevation is continuously measured using an optical system in the aperture. These experiments are usually performed in time-elevation mode. In this method the suction is switched on and off a number of times and elevation is measured as a function of time, which allows measurement of visco-elastic characteristics of the skin, two types of responses are obtained in this way, an immediate (elastic) and delayed (non-elastic) portion (6).

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Emulsions can offer promising applications in pharmaceutical, food and cosmetic industries. There has been renewed interest in the emulsion as a vehicle for delivering drugs to the body as it has been found to have several advantageous characteristics, frequently enhancing the bioavailability of the drug substance. In an emulsion, the therapeutic properties and spreading ability of the constituents are increased (7).

Calendula officinalis has shown to stimulate physiological regeneration and epithelisation of wounded skin (8). Being miscible with water, the extract was incorporated in internal aqueous phase of W/O cream. The aim of this study was to measure the effects of a W/O cream of *Calendula officinalis* extract on mechanical parameters of skin like R0, R1, R2, R5, R6, R7, and R8.

MATERIALS AND METHODS

Chemicals

The extract of *Calendula officinalis* plants (collected from fields in the month of March) was prepared in the cosmetic laboratory of Pharmacy department, The Islamia University of Bahawalpur, Pakistan. Paraffin oil was obtained from Merck (Germany). Abil- EM 90 was purchased from Franken Chemicals (Germany), Lemon oil was purchased from the local market of Pakistan.

Instruments

The instruments used were Cutometer MPA 580, MPA 5 (Courage + Khazaka, Germany), Digital Humidity Meter (TES Electronic Corp., Taiwan), Electrical Balance (Precisa BJ-210, Switzerland), Homogenizer (Euro-Star, IKA D 230, Germany), pH-Meter (WTW pH-197i, Germany), Rotary evaporator (Eyela, Co. Ltd., Japan), SPSS 12.0 and Water Bath (HH .$S_{21\,4.}$, China).

Methods

Emulsions (Creams)

In this study the products studied were newly formulated W/O emulsions (*base* and *formulation*) which were found stable after evaluating for pH, electrical conductivity, centrifugation, phase separation, temperature stability tests at 8 ± 0.1°C (in refrigerator), 25 ± 0.1°C, 40 ± 0.1°C and 40 ± 0.1°C with 75% RH (in incubator) and physical characteristics i.e., color, creaming and liquefaction.

The W/ O emulsions (cream) were prepared by the addition of aqueous phase to the oily phase with continuous agitation. The oily phase was composed of paraffin oil and surfactant (ABIL EM 90). The aqueous phase consisting of water and *Calendula officinalis* extract in a concentration of 3% was prepared and added into the oily phase drop by drop. Stirring was continued at different speeds and about 2 to 3 drops of lemon oil were added during stirring to give good odor to the formulation. The *base* was prepared with the same method as was used for the *formulation*. The only difference was that the active drug i.e., *Calendula officinalis* extract was not included in the aqueous phase of the *base*.

Study protocol

One-sided blind study was designed with placebo control in the month of August. Twenty-one healthy human volunteers who signed the informed consent, with age range 24–35 years were selected. Male volunteers were included in this work as they were easily available with regular under control observations. All the readings were performed at 21 ± 0.1°C and 40 ± 2% relative humidity conditions.

The experiments were carried out on the cheeks of volunteers. On the first day, patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. Each volunteer was provided with two creams. One cream was *base* and the other one was *formulation* containing the active ingredients. Each cream was marked with “right” or “left” indicating application of that cream to the respective cheek. Every individual was instructed to come on weeks 1, 2, 3, 4, 5, 6, 7, and 8 for the skin measurements. This was a facial cream, which the volunteers had to apply on their cheeks daily at night before sleeping for a period of 8 weeks.

Ethical standards

This study was approved by the Board of Advanced Study and Research (BASR), The Islamia University of Bahawalpur and institutional ethical committee, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, in compliance with NIH Principles of Laboratory Animal Care 1985 (Reference No. 1457/07). Recruitment for the study was aimed at male non-smoked, insensitive, aged 20–35 years. Prior to the tests, the volunteers were examined by a skin expert (dermatologist) for any serious skin disease or damage especially on cheeks and forearms. Before the study, every volunteer was provided and signed a volunteer protocol. This protocol was a bilingual consent document stating the terms and conditions like purpose, duration, risks, required procedures and potential benefits of the testing. Volunteers were not informed about the contents of formulations.

Skin elasticity measurements

Mechanical properties of the skin were determined with non-invasive suction skin elasticity meter (Cutometer MPA 580). A 2 mm diameter measuring probe was used, which applied a constant suction of 350 mbar with time/strain mode (Mode 1) for 18 s followed by a relaxation time of 2 s, with two repetitions. Measurements were made on a single site at face. The cutometer generated a graph depicting immediate deformation or skin extensibility (Ue), delayed distension (Uv), final deformation (Uf), immediate retraction (Ur), total recovery (Ua) and residual deformation at the end of measuring cycle (R) (Figs. 1 and 2). Parameters of curves of skin deformation generated by cutometer were then mathematically and statistically analyzed.

The first part of the curve is considered as the elastic component and is mentioned as Ue, whereas the second part of the curve characterizes the viscoelastic part of the skin, mainly the plastic component and is represented as Uv. The maximum amplitude of the curve Uf consists of: Uf = Ue + Uv (Table 1).

After the suction phase, the vacuum in the probe is automatically switched to 0 mbar of negative pressure. Here the curve dropped, and in the viscoelastic materials like skin, two parts of the curve can be seen. The perpendicularly dropping elastic component Ur and the viscoelastic component which is mathematically equal to Ua – Ur, whereas Ua is the total recovery after the suction phase (Table 1 and Fig. 2).

Table 1. The R-parameters given by the cutometer.

R0 = Uf = e(a)
R1 = Uf – Ua = e(a + b)
R2 = Ua / Uf = (e(a) – e(a + b))/e(a)
R3 = last max. amplitude = e((r × a) + ((r – 1) × b))
R4 = last min. amplitude = e((a + b) × r)
R5 = Ur / Ue = (e(a) – e(a + 0.1))/ e(0.1)
R6 = Uv / Ue = (e(a) – e(0.1))/e(0.1)
R7 = Ur / Uf = (e(a) – e(a + 0.1))/e(a)
R8 = Ua = ((e(a) × a × 100/ f(a) – 1) .100
R9 = R3 – R0 = e((r × a) + ((r – 1) × b)) – e(a)

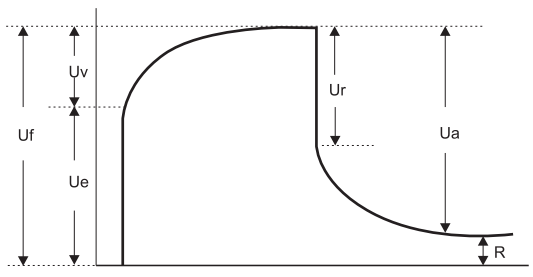

Figure 1. Skin deformation curve obtained with a cutometer.

Mathematical analysis

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

Percentage change = [(A – B) / B] × 100

Where A = individual value of any factor of 1st, 2nd, 3rd, 4th, 5th, 6th, 7th or 8th week and B = zero hour value of that factor.

Statistical analysis

The measured values obtained for different factors (R0, R1, R2, R5, R6, R7 and R8) were analyzed using SPSS 12.0 on the PC (paired sample *t*-test for variation between the two preparations; two-way ANOVA for variation between different time intervals). A 5% level of significance was used.

RESULTS

The values of R parameters following the application of the *base* and *formulation* on the cheeks of human volunteers have been presented in Figures 3–9.

DISCUSSION

Factor R0

R0 is known as the first maximum amplitude or the highest point of the first curve i.e., Uf. It is called a final distension or the skin distensibility (3). This has an implication for the firmness of the skin. It represents the passive behavior of the skin to force. In this study, it was found that after application of *base* there was an increase in R0 values in the 1st to 4th week but then the values decreased in 5th, 6th,

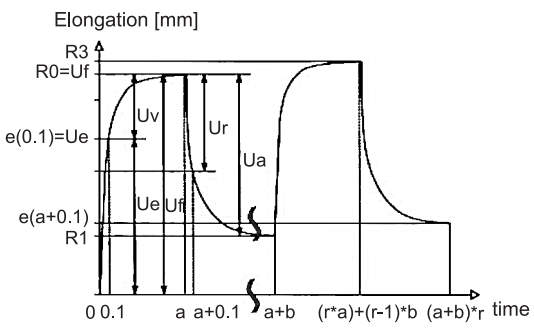

Figure 2. Derivation of the R factors from the curve

7th and 8th week (Fig. 3). After the application of *formulation* there was an increase in R0 values in the 2nd week but decrease was seen at all other times in the study, with the lowest values at the end of the study period i.e., in 8th week (Fig. 3). With the help of ANOVA test, it was found that changes in R0 values produced by *base* were insignificant during a period of 8 week study but significant with respect to time in the case of *formulation*. These changes were significant in 4th, 5th, 6th, 7th and 8th week.

R0 is an absolute parameter that is dependent on skin thickness and as the thickness of stratum corneum increases, there is an increased chance of a reduction in flexibility of the skin (12). R0 (Uf) and (Ue) is linked with the stretching of both collagen and elastic fibers and is inversely proportional to their thickness and rigidity (9, 10). So the *formulation* has the property of increasing the skin thickness as decreased R0 represents increased skin thickness. The usual approach of improving the skin is to soften the skin by the help of plasticizers or with the help of the occlusive effect of hydrophobic materials like oils and emollients. By this approach, the stress between the stratum corneum and the underlying epidermis and dermis is reduced because lines and wrinkles of the skin are smoothened. But a different approach is to bring about changes in the properties of the skin to induce tightness in the skin, which is also known as skin firming or skin lifting (11). The results show that this type of skin tightness can be induced by our *formulation*.

Factor R1

R1 represents the first minimum amplitude or it is also known as the lowest point of the first curve. It represents the ability of the skin to return to its original state (Uf – Ua) (12). In this study, it was found that values of R1 kept on changing throughout the study period after the application of *base* (Fig. 4). After application of *formulation* an increase in the values was seen on 2nd week but gradual decrease was seen at all other times (Fig. 4). With the help of ANOVA test, it was found that changes in R1 values produced by *base* as well as *formulation* were insignificant with respect to time. By individually analyzing the results it was seen that the 8th week values of the *formulation* showed significant decrease, so it may be assumed that if the study period is increased, the R1 values may further decrease, and the changes may become significant.

The decrease in R1 values shows the improvement of the skin to revert back to its original position after the application of stress, as mathematically R1 = Uf – Ua. The formulation does show improvement in the ability of the skin to revert back to its original state after the application of *formulation* during all the times except the 2nd week but overall statistically the changes are insignificant. In this study, Uf – Ua (R1) has decreased, so we may say that some improvement in skin to revert back to its original position after deformation was seen after application of *formulation*.

Factor R2

It is referred to as the gross-elasticity of the skin including the viscous deformation, and is represented by the ratio of "the ability of redeformation of skin" to "final distension" i.e., Ua/Uf (5) or it is also known as the overall elasticity of the skin including creep and creep recovery (13, 14). The

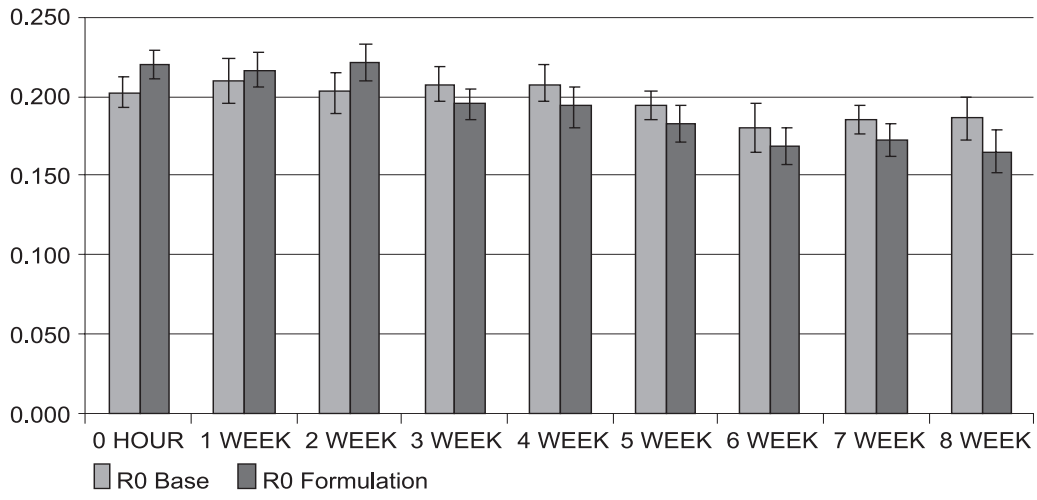

Figure 3. Values of the R0-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

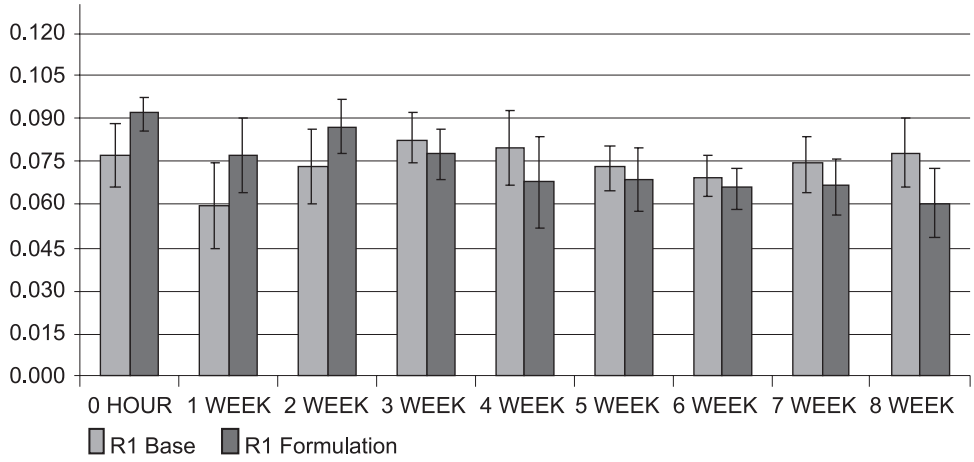

Figure 4. Values of the R1-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

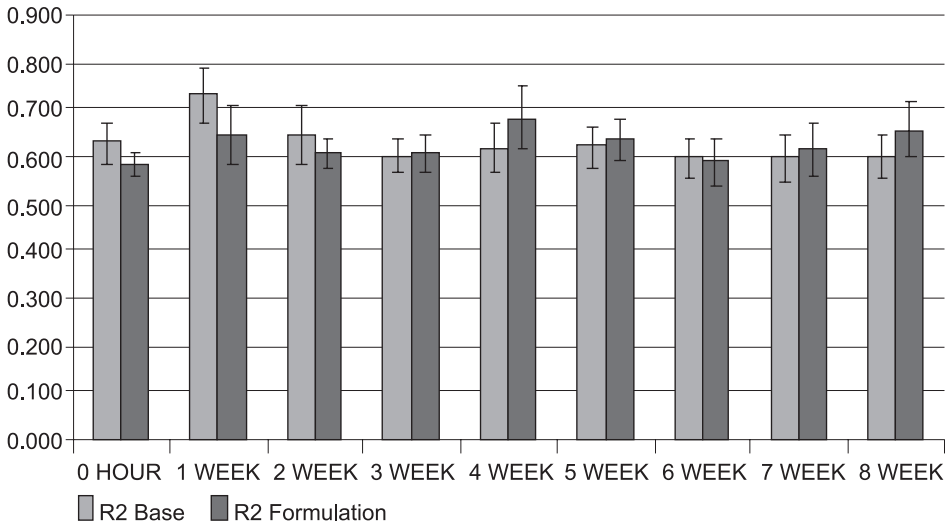

Figure 5. Values of the R2-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

closer the value is to 1 (100%), the more elastic the skin is. The parameter R2 together with parameter R7 is used as the main parameter to assess the skin elasticity and aging (3). Skin parameters are broadly divided into two main categories of elastic parameters and viscoelastic parameters, which are further subdivided into absolute and relative parameters. Ua/Uf is a relative elastic parameter which, like the Uv and Ur/Uf, measures the ability of the skin to return to its original position after deformation. This parameter is related to the function of the elastic fibers of the skin (10).

In this study, it was found that values of R2 increased in the 1st week of study, but then gradually decreased throughout the study period with some variations after the application of *base* and finally finished at values slightly less than the 0 hour readings (Fig. 5). With the application of *formulation* there was an increase seen in the 1st, 4th, 7th and 8th week (Fig. 5). With the help of ANOVA test, it was found that changes in R2 values produced by *base* as well as *formulation* were insignificant with respect to time. Therefore it may be stated that the *formulation* does not significantly improve the overall elasticity of the skin (R2).

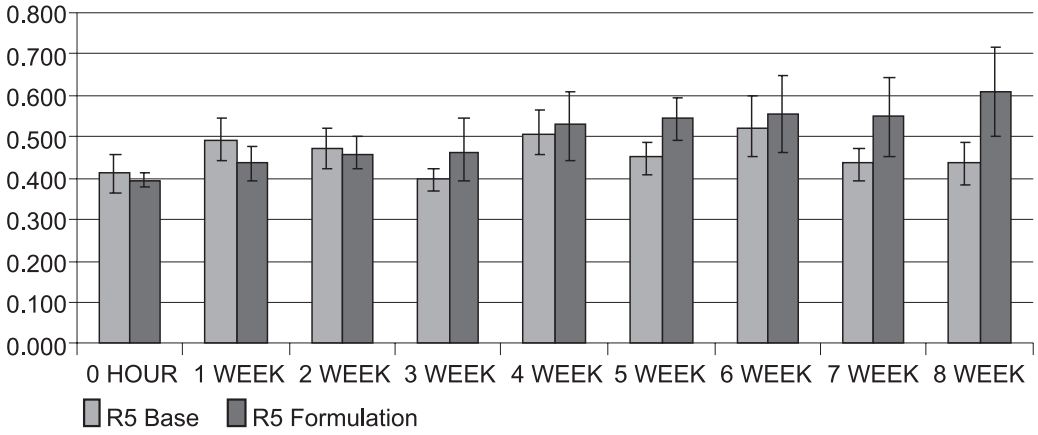

Figure 6. Values of the R5-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

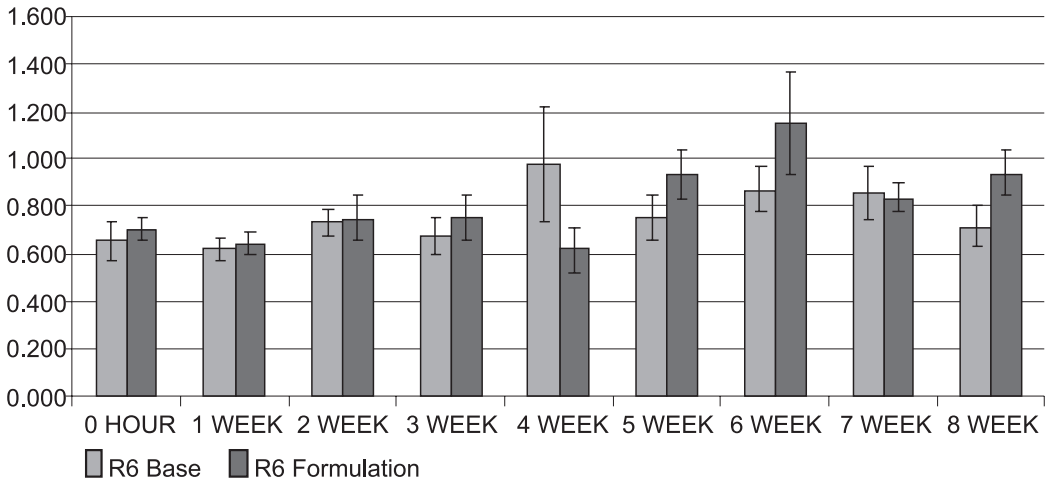

Figure 7. Values of the R6-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

Factor R5

It is, so called, the net-elasticity of the skin without the viscous deformation. Elastic recovery is represented by the ratio of "the immediate retraction" to "immediate distension" i.e., Ur/Ue (3, 5). The closer the value is to 1 (100%), the more elastic the skin is. The ratio of Ur/Ue is the parameter of choice for quantifying skin aging, as it represents the elastic recovery (ability of the skin to recover after deformation) independent of the thickness of skin. As the immediate recovery decays with age, so the index of elasticity (Ur/Ue) decreases with aging (15).

In this study, it was found that after application of *base* there was an increase in R5 values in the 1st, 4th, 6th, and 8th week but a decrease was observed at all other times, the 8th week values were not much higher than the 0 hour values (Fig. 6). After application of *formulation* there was continuous increase in R5 values throughout the study period with the maximum values seen at the end of the study period (Fig. 6). With the help of ANOVA test, it was found that changes in R5 values produced by *base* as well as *formulation* were statistically insignificant during a period of 8 week study. But if we compare 0 hour

values with 8th week values, a definite increase is seen which suggests that if we increase our study period the results may become significant.

Previous studies have shown a negative correlation of age with R2, R5 and R7 (12). Our *formulation* has also caused a decrease in these parameters (Figs. 5, 6 and 8), so this *formulation* may also delay the process of aging if used for longer periods of time.

Factor R6

It is the portion of the viscoelasticity on the elastic part of the curve. It indicates the relative contribution of viscoelastic plus viscous deformation to elastic deformation of the skin and is attributed to the displacement of interstitial fluid throughout the fibrous network (9, 12). It is represented by the ratio of "viscoelastic" to "elastic distension" i.e., Uv/Ue. This parameter is the most indicative of epidermal and dermal water content (5). This is because the mechanical and the electrical properties of the skin are affected by the skin's hydration level. Especially, the plasticity and electrical capacitance of the stratum corneum are increased by the increased water content (13).

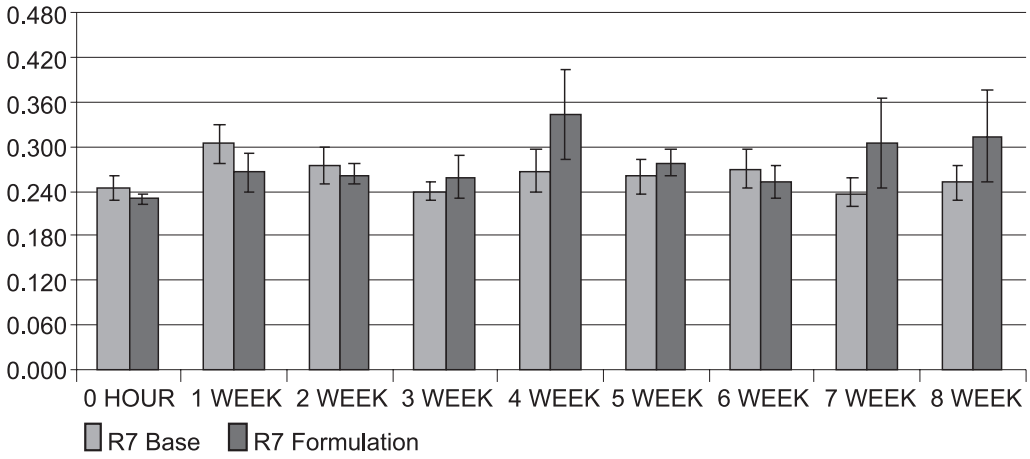

Figure 8. Values of the R7-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

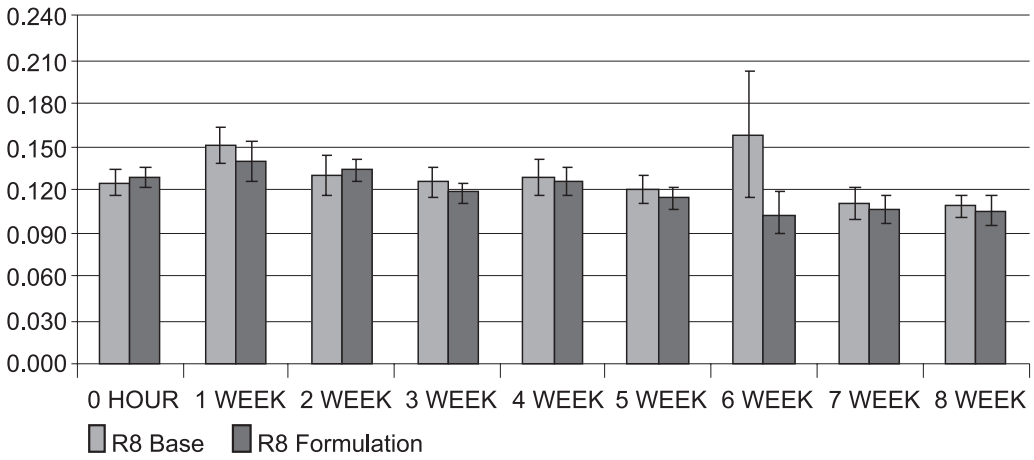

Figure 9. Values of the R8-parameter of all the volunteers at different time intervals after application of *base* and *formulation*

In this study, it was found that after application of *base* there was a major increase in R6 values in the 2nd, 4th and 6th week, while at other times it decreased (Fig. 7). On the other hand, the values increased at all times after the application of *formulation* except in the 1st, 4th and 7th week (Fig. 7). With the help of ANOVA test, it was found that changes in R6 values produced by *formulation* were significant during a period of 8 week study, while in the case of *base* these values were insignificant. The increases in the relative parameter R6 may indicate a decrease in interstitial fluid viscosity because of the result of an increased water content thereby suggesting an increase in skin hydration. Such hydration effect is very important for normal cutaneous metabolism and also may prevent skin alteration and early ageing (16).

The significant increase in viscoelasticity after *formulation* may be attributed to the constituents present in the calendula extract, like carotenoids (17), saponins, glycosides of sesquiterpenes, flavonoids and triterpenes etc. (18). Such property has also been seen in a previous study, in which a derivative of ascorbic acid – magnesium ascorbyl phosphate (MAP) which possesses antioxidant property also increased the Uv/Ue parameter. It also correlates with another study in which the skin was slightly dehydrated by using an alkaline soap and the results revealed a decrease in both Ur/Ue (R5) and Uv/Ue (R6) parameters (16). In another study Dobrev have shown a positive correlation between epidermal hydration and the parameters R5 and R6 (5).

Factor R7

It is referred as the biological elasticity (19). It is the portion of elasticity compared to the final distension. It is represented by the ratio of "the immediate retraction" to "final distension" i.e., Ur/Uf (5). The closer the value is to 1 (100%), the more elastic the skin is.

In this study, it was found that after application of *base*, the values of R7 increased markedly in the 1st week of study, and appreciable increase in the 4th 6th and 8th week was seen, but a decrease was seen at other times (Fig. 8). An increase was observed in the 1st, 4th, 7th and 8th week after application of *formulation*, while a decrease was seen at other times (Fig. 8). With the help of ANOVA test, it was found that changes in R7 values produced by *base* as well as *formulation* were insignificant with respect to time. However, the regular increase after the 6th week with the application of *formulation* (Fig. 8) suggests that some improvement in biological elasticity of skin has been produced by *formulation* although statistically it is insignificant. It is expected that by increasing the study period a clearer picture of the effects of *formulation* on this parameter can be obtained.

Factor R8

It is equal to Ua (total recovery) of the first curve (12). It is the viscopart i.e., the area under the suction part of the deformation curve (3) or the viscoelastic character (20). It is also known as the pliability of the skin and is defined as the difference between the maximum deformation and the deformation after one second of normal pressure (21). The closer Ua and Uf, the greater is the ability of the skin to return into its original state.

In this study, it was found that values of R8 after application of base increased in the 1st, 4th and 6th week of study, but decreased at other times (Fig. 9). After the application of *formulation* there was a significant increase in R8 values in the 1st, 4th and 7th week and a decrease at all other times (Fig. 9). With the help of ANOVA test, it was found that changes in R8 values after application of formulation and base were insignificant with respect to time.

The normal skin usually has the viscoelastic character (R8), around 0.1 (20). In our study, the volunteers initially had higher R8 values which generally decreased during the study. In case of *formulation*, at the last three times (6th, 7th and 8th week), the values were 0.103, 0.106 and 0.105, respectively (Fig. 9), which are very near to 0.100, so we can say that our formulation may have the ability to bring the higher viscoelastic character values towards the normal.

CONCLUSION

Calendula officinalis is a herbal plant whose effects on the skin have not been studied extensively. Our study has shown that a cream containing *Calendula officinalis* can produce some valuable effects on the skin. In this study it was found that the *formulation* had the ability of inducing skin tightness which prevents the damage of skin and also delays the aging process. The *formulation* also increased the hydration of the skin and such hydration effect is very important for normal cutaneous metabolism and may also prevent skin alteration and early ageing. It also showed some improvement in some elastic and viscoelastic parameters though it was not significant. Our study was just an initial step to find the valuable effects of the plant and further studies in this area should be continued by changing the concentration of the extract in *formulation* and by incorporating the volunteers who are aged or are having some skin disease.

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REFERENCES

1. Dal'Belo S.E., Gaspar L.R., Gonçalves Maia Campos P.M.B.: Skin Res. Technol. 12, 241 (2006).
2. Hunter J.A.A., Savin J.A., Dahl M.V.: Clinical Dermatology, Blackwell Science, London 2002.
3. Ahn S., Kim S., Lee H., Moon S., Chang I.: Skin Res. Technol. 13, 280 (2007).
4. Lagarde J.M., Rouvrais C., Black D.: Skin Res. Technol. 11, 110 (2005).
5. Dobrev H.: Skin Res. Technol. 6, 239 (2000).
6. Schlangen L.J.M., Brokken D., Van Kemenade P.M.: Skin Res. Technol. 9, 122 (2003).
7. Lieberman H.A., Rieger M.M., Banker G.S.: Pharmaceutical Emulsions, Pharmaceutical Dosage Forms: Disperse Systems, Vol 1, Marcel Dekker, New York 1988.
8. Barnes J., Anderson L.A., Phillipson D.J.: Herbal Medicines. 2nd edn., Pharmaceutical Press, New York 2002.
9. Hashmi F., Malone-Lee J.: Skin Res. Technol. 13, 252 (2007).
10. Dobrev H.: Skin Res. Technol. 13, 91 (2007).
11. Dobrev H.: Skin Res. Technol. 4, 155 (1998).
12. Jachowicz J., McMullen R., Prettypaul D.: Skin Res. Technol. 14, 312 (2008).
13. Ryu H.S., Joo Y.H., Kim S.O., Park K.C., Youn S.W.: Skin. Res. Technol. 14, 354 (2008).
14. Murray B.C., Wickett R.R.: Skin. Res. Technol. 3, 101 (1997).
15. Koch R.J., Cheng E.T.: Arch. Facial. Plast. Surg. 1, 272 (1999).
16. Maia Campos P.M.B.G., Gonçalves G.M.S., Gaspar L.R.: Skin Res. Technol. 14, 376 (2008).
17. Adela Pintea A., Constantin Bele C , Sanda Andrei S., C. Socaciu C.**:**: Acta Biol. Szeged 471, 37 (2003).
18. Petrović L., Lepojević Ž., Sovilj V., Adamović D., Tešević V.: J. Serb. Chem. Soc. 72, 407 (2007).
19. Paye M., Mac-Mary S., Elkhyat A., Tarrit C., Mermet P., Humbert P.H.: Skin Res. Technol. 13, 343 (2007).
20. Smalls L.K., Wickett R.R., Visscher M.O. Skin Res. Technol. 12, 43 (2006).
21. Fong S.S.L., Hung L.K., Cheng J.C.Y.: Burns 23 (Suppl.), 12 (1997).

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