International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1328-1343www.ijprajournal.comISSN: 2249-7781

In vivo Skin Irritancy Study of a Novel Anti-Aging Skincare Regimen: A Single Blinded Human Volunteer Study for Safety Evaluation.

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Submitted: 10-05-2023 Accepted: 23-05-2023

ABSTRACT: Vitamin A and its derivatives, also known as Retinoids, are proven to be the most effective topical anti-aging treatments; however, skin irritation is considered as a major and often adverse effect, making retinoids a difficult treatment option for both medical professionals as well as patients. Before applying any topical products that contain retinoids, it is conventionally advised to perform a patch test to observe the skin irritancy. The severity of skin irritation varies with skin types, types of retinoid analogues, and the total strength of retinoids used in a topical dosage form. Although the minimal effective topical dose of retinoids (0.1%) exerts a certain level of skin irritation and skin dryness, a novel skincare regimen for anti-aging called "EI Pro Retinol Series" is aimed and designed to reduce the severity of irritation by formulating with appropriate base ingredients and other compatible active ingredients with calming and soothing benefits. In this current study, we evaluated the skin irritancy potential of the "EI Pro Retinol Series", a complete treatment regimen that contains three different retinoid serums, a moisturizer, and a sunscreen that are developed for sensitive and dry skin conditions. Three topical serums were formulated with different combinations of retinoid derivatives, named Alpha, Beta, and Gamma, with total retinoid strengths of 0.14%, 0.67%, and 1%, respectively. Along with topical serums, a moisturizer was formulated to be used after serum application to counter dry skin conditions and to enhance the efficacy of serums. A non-greasy & non-irritant sunscreen suitable for sensitive skin with SPF 50+ and PA++++ was also developed to shield the sensitive skin from harmful ultraviolet radiation. A single-blinded study on healthy human volunteers was done to test for skin irritation potential using the single application patch test method. In this study, dermatologists observed that the total mean score of irritation on T2 day was 0.0 (24 hours after patch removal). The negative control (0.9% isotonic saline solution) showed no reaction, which was compared with a 2.1 mean score for the positive control (1% w/w SLS solution). For Alpha, Beta, and Gamma serums, the total score of erythema and oedema was 0.0, whereas in Moisturizer and sunscreen formulations, the total score of erythema was 1.0 and oedema was 0.0. The synergistic combinations of calming and soothing actives, an anti-ageing peptide, and vitamins might have helped in achieving zero irritancy. Hence, this anti-aging skincare regimen, "EI Pro Retinol Series" has proved to be one of the safest anti-aging regimens in the modern cosmetic industry.

Key Words: Skin irritation, Patch Test, Retinoids, Anti-aging, Topical Retinoid Treatment.

I. INTRODUCTION:

Researchers suggest that Retinoids play a crucial role in diminishing wrinkles, fine lines, age spots, scars, and photoaging.(1) Retinoids stimulate keratinocyte proliferation, restore the skin barrier, boost collagen synthesis, and regulate trans-epidermal water content.(2) Since Retinoic acid (Vitamin A) in purest form is difficult to stabilize in cosmetic topical applications, usually other analogue chemical derivatives such as alcohol, aldehyde, and esters, along with encapsulated forms, are widely used to enhance efficacy and stability. Most of the recent anti-ageing cosmeceuticals are formulated with an individual or combination of Retinol (aldehyde), Retinol

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1328-1343www.ijprajournal.comISSN: 2249-7781

liposomes (alcohol and encapsulated form), retinyl palmitate (ester), and hydroxypinacolone retinoate (ester).[3,4]

Retinoids work by binding to retinoic acid receptors (RAR-α, -β, -γ) as well as retinoid-X receptors (RXR- α, -β, -γ).[5] Initially, Retinyl esters are broken down into Retinol, which is then transformed into active retinoic acid via Retinol in a 2-step oxidation process mentioned in Figure 1. The retinoic acid is transformed into all-trans retinoic acid (ATRA) by binding to the in vivo retinoic acid receptors mentioned in Figure 2.[6] According to recent research published in 2022, it has been proven that the antiaging efficacy order of retinoids is as follows: retinyl palmitate < Retinol < Retinol < retinoic acid. However, the order of tolerance is vice versa: retinyl esters > Retinol > Retinol > retinoic acid.[7] Based on the earlier mentioned research data, a new anti-aging skincare regimen called ‘‘EI Pro Retinol Series’’ was designed with a combination of the aforementioned retinoid derivatives & desired to have reduced irritancy and higher safety profile. The ‘‘EI Pro Retinol Series’’ was designed with a variety of tried-and-true naturals, vitamins, and peptides for more calming, moisturizing, and revitalizing effects and are as mentioned in Table 1.

Three topical serums were formulated with different combinations of Retinol derivatives, named Alpha, Beta, and Gamma, with total retinoid strengths of 0.14%, 0.67%, and 1%, respectively. In Alpha serum a dual combination of retinal (aldehyde) and Retinol (alcohol) liposomes were used. Beta serum contains a triple combination of hydroxypinacolone retinoate (HPR) (ester), retinal (aldehyde) and Retinol (alcohol) liposome. Gamma serum includes a triple combination of retinyl palmitate (ester), retinal (aldehyde) and Retinol (alcohol) liposomes. Thus, three unique combinations of retinoids were set for three different serums according to the strength and efficacy. In Alpha serum, a combination of Retinal and Retinol Liposomes were used as Retinal aids in photoaging, helps in the treatment of fine lines and wrinkles and causes less irritation when compared to retinoic acid.[8] Retinol Liposomes increase the thickness of the epidermis, increases collagen production, and thus helps in skin elasticity and reduces fine lines, wrinkles, and uneven skin tone.[9,10] Retinol, on the other hand, is broken down by air, light, and heat and causes erythema. Retinol is better protected and distributed to the skin when it is encapsulated in liposomes. Thus, a dual combination of Retinol and Retinol liposome were chosen for Alpha serum with a total retinoid strength of 0.14%. In Beta serum, a triple combination of HPR, Retinal & Retinol liposomes are used and contain a total retinoid strength of 0.67%. As per the RARE assay by Ruth et al. in 2018, the novel cosmetic-grade retinoid called “HPR” mimics trans-retinoic acid's wrinkle-reducing action without causing itching and irritation, and it was also proved to boost procollagen biosynthesis. The results showed that HPR had greater levels of gene transcription than Retinol liposomes, Retinol, and Retinyl palmitate at the same concentrations and was less cytotoxic to skin cells at 10 times the normal concentration.[11] It was shown to be more stable and less irritating to the skin than Retinol. HPR demonstrated superior anti-wrinkle and skin elasticity enhancement while being well tolerated in combination with other retinoid derivatives.[4] Retinal and Retinol liposomes advantages are already listed above; Thus this triple combination was used to formulate Beta serum with a total of 0.67% of retinoid strength. In Gamma serum, Retinyl palmitate was used and it was proved to substantially increase the thickness of the outer skin layer (epidermis), helps in collagen synthesis, and is also an effective anti-wrinkle agent.[12] Vitamin A ester (palmitate) has been preferred over Retinol and retinoic acid because of its superior chemical stability and safety profile.[10] As mentioned earlier, Retinal & Retinol liposomes were used along with Retinyl Palmitate with a total retinoid strength made to 1%.

The higher the Retinol concentration, the greater the efficacy; hence, the stages of Alpha, Beta, and Gamma were assigned in accordance with the increasing order of total retinoid strength. In addition, to avoid unnecessary chemical interactions between retinoids and other synergistic ingredients and to improve the stability of retinoids, synergistic ingredients were included in a moisturizer that is recommended for use after serum application. Furthermore, a non-greasy, aqueous-based sunscreen was formulated, which shields sensitive, dry skin from harmful ultraviolet radiation. To evaluate erythema and oedema caused by retinoids, we developed a single-blinded in vivo patch test method involving human volunteers.

II. METHODS:

‘‘EI Pro Retinol Series’’ comprises a Sunscreen, Moisturizer, Alpha serum, Beta serum, Gamma serum coded as A, B, C, D and E respectively, as clearly shown in figure 3. The skin

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1328-1343www.ijprajournal.comISSN: 2249-7781

sensitization study of the above ‘‘EI Pro Retinol Series’’ is done by the single application patch test method mentioned in Table 2.[13]

Study design protocol:

Based on inclusion and exclusion criteria, 12 women and 12 healthy men volunteers were chosen between 18 and 54 years of age for a single-site, non-randomised trial in which a closed, occlusive patch was given. For a total of 8 days, participants served as their own best resource [3 days, with a follow-up visit set on T8 (T+1 week after 0 hours of patch removal)]. Before the research, the principal investigators fill out a questionnaire about their medical history, allergies, cosmetics habits, research methodologies, practical challenges, finances, volunteers' aesthetic advantages, and obstacles to volunteers. Participants read and signed the consent form for the research about accessibility throughout the study. No one was convicted, hospitalized without consent, or admitted to an institution.

Inclusion criteria:

The study was conducted on subjects who fulfilled the criteria like female and male healthy Asian Indian subjects with no infectious or evolutive pathology, no symptoms in the process of an exploratory check-up, and subjects between 18 and 65 years of age free of eczema, wounds, inflammatory scars, etc.

Subjects were screened with the following non-inclusion criteria: pregnancy, breastfeeding, or recently stopped breastfeeding for the past 3 months, diabetes, asthma, aspirin-based medications, anti-inflammatories, antihistamines, corticotherapy, dermatitis, allergies to cosmetic components, and post-surgery with more than an hour of general anaesthesia. They were excluded from this study. Changes in cosmetic habits prior to 14 days before the study were not allowed, and no makeup was permitted on the day of patch application. Subjects taking part in other studies were not considered in this research.

Specific non-inclusive criteria: Participants diagnosed with eczema, psoriasis, lichen planus, vitiligo, rhinitis, conjunctivitis, rhinosinusitis, plaster or food allergies, having cardiac pathology (taking a beta-blocker treatment), immunosuppressants, and retinoids taken orally or via the general route were not taken in this study. Subjects with perfume or preservative allergies, treatment involving antihistamines, and within two weeks before the trial begins, having "prickly heat" (miliaria) on the back, multiple back nevi, or having high pilosity on the back were excluded from this study.

Restrictions to follow during the study: Cosmetics or water were not applied for 24 hours after the patch test. From T2 to T8, only water was accepted from the first reading (24 hours after the patch removal). The sun on the back was avoided.

Patch preparation for test products: The patches were prepared in the morning of the application, one hour before the first visit, i.e., at 20°C - 25°C at T0. The items were stored in an IP storage room (temperature: 20 - 25°C, humidity: 40 - 60% RH).[14]

Procedure for patch preparation of products A, B, C, D, and E as per BIS Standard Clause 4.3.1.2, IS 4011:2018, 3rd Revision: 0.04 (40tl) of test samples were measured with a syringe. The test products were transferred with a 1 ml syringe onto a previously numbered Aluminium Finn chamber. Subjects' backs were taped with a loaded Finn chamber.

Patch preparation for negative control as per BIS Standard clause 4.3.1.2.4, IS 4011:2018, 3rd Revision: 40 µl of 0.9% isotonic saline solution was transferred to the previously numbered Aluminium Finn Chamber with a Whatman No. 3 filter paper with a micropipette. Finn chambers with 0.9% isotonic saline solution were taped to the backs of the subjects.

Patch preparation for positive control as per BIS Standard clause 4.3.1.2.4, IS 4011:2018, 3rd Revision: 1% w/w SLS solution in distilled water was made. 0.04 ml or 40 µl of SLS solution was deposited on a Whatman No. 3 filter paper disc in a clean Aluminium Finn Chamber. The Finn chamber with 1% w/w SLS was taped to the backs of subjects.

Methodology of patch application: The dermatologist chose the location on the body based on moles, body hair, and freckles without excessive hair growth between the scapula and waist, as shown in Figure 4. When the strip was put on from the bottom, the rooms were pushed up to let the air out. When the tape was in place, each test product

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1328-1343www.ijprajournal.comISSN: 2249-7781

or control patch was gently pressed with micropore tape to ensure even distribution and good fixation.

Dermatological evaluation procedure: The patch test under occlusion checks the irritation potential of cosmeceutical formulations applied topically on healthy humans. The nature, concentration, and duration of the exposure will determine the extent of the harm. Irritation manifests as inflammatory responses such as erythema and oedema, vesiculation, and a severe suppurative reaction without the immune system. The products (A, B, C, D, and E) were evaluated against a positive control: 1% (w/w) sodium lauryl sulfate and a negative control: 0.9% isotonic saline solution.

The kinetics of the evaluation were as follows: T0 = (before patch application) Patch application, T1 day = (0 hr after the patch removal) Patch removal, T2 days = (24 hours after patch removal) Patch reading by the dermatologist: T8 = (T+1 week after 0 hours of patch removal) Checking the evolution of the positive cases.

Study areas and locations: The patches were applied on the top of the back, near the shoulder blade. The dermatologist determined the best area to apply the patch, depending on the nevi, pilosity, and freckles. The selected area was healthy without excessive pilosity. The patches applied to the right or left part of the back were mentioned in the case report form (CRF) by the dermatologist.

Methodology of patch removal: The clinical research associate (CRA) removed the patch from the bottom to the top and gently wiped it with a soft tissue paper.

Methodology of patch reading by the dermatologist: Skin response was evaluated under artificial daylight. According to the Draize Scale, the dermatologist scored erythema (dryness, scaliness, and wrinkles) and oedema on a 0–4-point scale (Clause 4.3.1.3 Observation and Scoring for Skin Irritation Test, Draize Scale for Scoring the Treatment Sites, IS 4011:2018 Methods of Test for Safety Evaluation of Cosmetics, 3rd Revision), which was mentioned in Table 3. [15]
On T2 day, patches were photographed if a positive reaction was noticed. Another shot at T+8 days is evaluated to show the signs of progression.

EXAMINATION SCHEDULE: The effect of products were examined over 3 days and at T8 (T+lweek after patch removal). At T0 (before patch application) and Tl (one hour after patch removal), acclimatization is performed for 20 minutes at a temperature of 20°C–25°C, with subsequent prescriptions, restrictions, and concomitant medication, followed by a dermatological evaluation, a patch test reading, and serious adverse event monitoring. On T2, the same technique is followed (24 hours after the patch removal). However, at T8 (T+l week following patch removal), the identical procedure was repeated, with the dermatologist filling out an additional study completion form.

Data Analysis and Statistics of Technical Data: Data analysis was carried out by the study in-charge. The assessment was only based on the mean score obtained 24 hours after the patch removal (T2).

The mean score for irritation =

$$\frac{\text{Total score (Erythema + Oedema) for each sample}}{\text{Total number of subjects}}$$

According to Clause 4.3.1.3, Observation and Scoring for Skin Irritation Test, the Draize scale for scoring the treatment sites- IS 4011:2018 (Methods of Test for Safety Evaluation of Cosmetics - 3rd Revision) the mean score of observations was calculated for measuring skin irritation at the investigational site. The mean score of 2.0 out of 8.0 is considered non-irritant; up to 4.0 out of 8.0 is considered a mild irritant; and any score above 4.0 to 8.0 is considered an irritant.[15]

Ethical and Legal Considerations: The study was done, and the insurance number is (121200/48/2022/7027). All subjects signed their consent forms after proper understanding. The quality system was in full compliance with ICH-E6 Good Clinical Practise (GCP) guidelines in the test companies. This study followed the latest guidelines of the World Medical Association (Declaration of Helsinki, 1964, revised in Fortaleza, Brazil, 2013) according to the "Drugs and Cosmetics Act Schedules".

Absences: Subject 024 was absent on the T+1 day visit. As the interpretation of results was based on a T+2 day visit, hence the data of the subject was not exploited in the global study results.

Action taken: As per sponsor request, to complete the study for 24 subjects as per ‘IS 4011:2018

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1328-1343www.ijprajournal.comISSN: 2249-7781

Methods of Test for Safety Evaluation of Cosmetics, 3rd Revision, one additional subject 024 was enrolled on August 10, 2022, to complete the study for 24 subjects as per the BIS standard.

III. RESULTS:

The exploited panel consisted of 24 healthy women and men subjects aged between 18 and 54 years old of Asian (Indian) skin type. Dermatological evaluation was done as per the Draize Scale to study the parameters like erythema and oedema, which were already mentioned in Table 3. The total mean scores for erythema and oedema parameters and observation results at T2 days that were 24 hours after the patch removal were summarized in Table 4.

The dermatologist found no irritative response on T2 day (24 hours after patch removal). There was no reaction observed for the negative control (i.e., 0.9% isotonic saline solution), which was compared to the mean score of 2.1 for the positive control (1% w/w SLS solution). For formulations A, B, C, D, and E, the overall mean score for both erythema and oedema was 0.0, which was clearly shown in Graphs 1 and 2.

IV. DISCUSSION:

The ‘‘EI Pro Retinol Series’’ is an exclusive combination of Retinol and its derivatives with synergistic molecules such as vitamin C, hyaluronic acid, peptides, xylitol, allantoin, alpha-bisabolol, alpha arbutin, and saccharide isomerate. The serums contain alpha-bisabolol, which is listed in Table 1, which suppresses the production of pro-inflammatory cytokines and decreases skin inflammation, which is in accordance with results by Maurya et al. in 2014.[16] Allantoin was included in the serums to prevent erythema and oedema on the skin and to counteract the irritating effects of the retinoids and it also possesses promising anti-inflammatory potential according to the research conducted by Danica et al. in 2021.[17] Betaine was used in both serums & moisturizer and it reduces oxidative stress-induced inflammation and facilitates smooth and plump skin.[18] All three serums contain Cucumber, a super food, that is widely used to prevent a variety of skin-related problems like sunburn & redness and to soothe itchy and irritated skin.[19] Pentavitin, a patented product from DSM nutritional products containing Saccharide isomerate, is added to serums since it has a relatively sophisticated moisturizing activity than glycerine that lasts up to 72 hours; it exfoliates dead skin, soothes inflammation, and derives a feathery soft skin.[20] All serums contain Aloe vera, which is rich in mucopolysaccharides, essential amino acids, and zinc, all of which support the integrity of the skin, aid in the retention of moisture, and lessen erythema.[21] Entire regimen consists of Vitamin E as it has both antiaging and skin brightening benefits that help in wrinkle reduction and add a glow to the skin.[22] Palmitoyl tripeptide-5 played a great role in serums and moisturizer by replenishing collagen via activating TGF-beta and imitating the extracellular matrix protein thrombospondin-1.[23] Vitamin C is an antioxidant, an anti-inflammatory, protects the skin from oxidative stress, and aids in the treatment of erythema induced by ultraviolet radiation.[24] Thus, Ethyl Ascorbic acid, a stable form of Vitamin C was added to "EI PRO Retinol Series". Oligo sodium hyaluronate was used in ‘‘EI Pro Retinol serums’’ and a combination of high, medium, and low molecular weight hyaluronic acids in EI Pro Retinol sunscreen and moisturizer since very low molecular weight hyaluronic acids pierce the deepest layers of the skin and reduce fine lines and wrinkles, whereas high molecular weight hyaluronic acids regulate skin hydration and keep it plump, which is also in accordance with the results shown by Widgerow et al. in 2022.[25] Aquaxyl is a patented combination of xylitol, anhydroxylitol, and xylitylglucoside based on hydro concepts of seppic that traps free water molecules, rebuilds skin, and maintains natural skin hydrous flow; thus, aquaxyl was added in all the Serums.[26]Aquaxyl and allantoin were added in serums since they mitigate sunburn and photoaging-induced redness and swelling synergistically, this likely contributed to the silky, soft, and dewy aspect of the products, the same was stated by Worrall in 2020.[27] As allantoin, bisabolol, and aquaxyl have anti-irritancy properties, which were added to the serums, zero irritation was observed in all three serums. However, erythema of 1.0 was observed in sunscreen and moisturizer, the levels are within safe range and the overall mean score for both erythema and oedema was 0.0. Thus, ‘‘EI Pro Retinol regimen’’ is considered safe and non-irritant according to the Draize scale displayed in Table 3.

V. CONCLUSION:

The synergistic combination of calming and soothing ingredients, an anti-ageing peptide, and skin-nourishing vitamins might have

significantly contributed to the achievement of zero irritancy in the ‘‘EI Pro Retinol Series’’. Hence, the ‘‘EI Pro Retinol Series’’ is regarded as one of the safest anti-ageing regimens in the cosmetic industry.

Acknowledgements: We would like to thank Esthetic Insights, Jeedimetla, for research assistance and funding, as well as Mascot Spincontrol, Mumbai, for the help in conducting this research.

REFERENCES:

[1]. Sorg, O., Antille, C., Kaya, G. and Saurat, J.H. (2006) Retinoids in cosmeceuticals. Dermatologic Therapy, 19, 289-296. https://doi.org/10.1111/j.1529-8019.2006.00086.x

[2]. Arora, G. (2021) Insights into Cosmeceuticals. CosmoDerma, 1, 1-8. https:doi.org/10.25259/CSDM_34_2021

[3]. Rousselle, C. (2017) Opinion of the Scientific Committee on Consumer Safety (SCCS) – Final version of the Opinion on Vitamin A (retinol, retinyl acetate and retinyl palmitate) in cosmetic products. Regulatory Toxicology and Pharmacology, 84, 102-104. https://doi.org/10.1016/j.yrtph.2016.11.017

[4]. Wang, Q., Hu, X., Xie, Y. and Du, Le. (2023) The synergistic effect of retinyl propionate and hydroxypinacolone retinoate on skin aging. Journal of Cosmetic Dermatology.1–10. https://doi.org/10.1111/jocd.15662

[5]. Shin, J.W., Kwon, S.H., Choi, J.Y., Na, J.I., Huh, C.H., Choi, H.R. and Park, K.C. (2019) Molecular mechanisms of dermal aging and antiaging approaches. International Journal of Molecular Sciences, 20, 1-16. https://doi.org/doi: 10.3390/ijms20092126.

[6]. Ziouzenkova, O. and Plutzky, J. (2008) Retinoid metabolism and nuclear receptor responses: New insights into coordinated regulation of the PPAR–RXR complex. FEBS Letters, 582, 32–38.https://doi.org/10.1016/j.febslet.2007.11.081

[7]. Milosheska, D. and Roskar, R. (2022) Use of Retinoids in Topical Anti Aging Treatments: A Focused Review of Clinical Evidence for Conventional and Nanoformulations. Advances in Therapy, 39, 5351-5375. https://doi.org/10.1007/s12325-022-02319-7

[8]. Mukherjee, S., Date, A., Patravale, V., Korting, H.C., Roeder, A. and Weindl., G. (2006) Retinoids in the treatment of skin aging: An overview of clinical efficacy and safety. Clinical Interventions in Aging, 1, 327–348. https://doi.org/10.2147/ciia.2006.1.4.327

[9]. Aparajita, V. and Ravikumar, V. (2014) Liposomes as carriers in skin ageing. International journal of Current Pharmaceutical Research, 6, 1-7.

[10]. Zasada, M. and Budzisz M. (2019) Retinoids: active molecules influencing skin structure formation in cosmetic and dermatological treatments. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. Advances in Dermatology and Allergology, 4, 392-397. https://doi.org/10.5114/ada.2019.87443

[11]. Ruth N, Mammone T. (2018) Anti-aging effects of retinoid hydroxypinacolone retinoate on skin models. Journal of investigative dermatology, 138:S223. https://doi.org/10.1016/j.jid.2018.03.1327

[12]. Pyo, Y.H. and You, S.H. (2019) A Study on the Latest Research Trends in Natural Products with Anti-Aging Effects. Journal of Convergence for Information Technology, 9, 286-293. https://doi.org/10.22156/CS4SMB.2019.9.12.286

[13]. Sugiyama, M., Akita, M., Alepee, N., Fujishiro, M., Hagino, S., Handa, Y., Ikeda, H., Imai, N., Jitsukawa, S., Katoh, M., Kurihara, K., Kyotani, D., Nomura, S., Okamoto, Y., Okumura, H., Omori, T., Sugibayashi, K., Todo, H., Toyoda, A. and Ohno, Y. (2018) Comparative assessment of 24-hr primary skin irritation test and human patch test data with in vitro skin irritation tests according to OECD Test Guidelines 429 (for quasi-drugs in japan). The Journal of Toxicological Sciences, 43, 751-768. https://doi.org/10.2131/jts.43.751

[14]. Kanto, H., Washizaki, K., Ito, M., Matsunaga, K., Akamatsu, H., Kawai, K., Norito, K., Kawai, K., Katoh, N., Natsuaki, M., Yoshimura, I., Hajime, Kojima., Okamoto, Y., Okuda, M., Kuwahara, H., Sugiyama, M., Kinoshita, S. and Mori, F. (2013) Optimal patch application time in the evaluation of skin irritation. The Journal of Dermatology, 40, 363–369. https://doi.org/10.1111/1346-8138.12004

[15]. Draize, J.H., Woodard, G. and Calvery, H.O. (1994) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. Journal of Pharmacology and Experimental Therapeutics, 82, 377-390.

[16]. Maurya, A. K., Singh, M., Srivastava, S. and Luqman, S. (2014) α-(-)-Bisabolol Reduces Pro-Inflammatory Cytokine Production and Ameliorates Skin Inflammation. Current Pharmaceutical Biotechnology, 15, 173-181. https://doi.org/10.2174/1389201015666140528152946.

[17]. Dinica, R.M., Sandu, C., Botezatu, A.V.D., Busuioc , A. C., Balanescu, F., IonicaMihaila, M.D.L., Dumitru C, N., Furdui, B. and Iancu, A.V. (2021) Allantoin from Valuable Romanian Animal and Plant Sources with Promising Anti-Inflammatory Activity as a Nutricosmetic Ingredient. Sustainability; 13, 1-17. https://doi.org/10.3390/su131810170.

[18]. Arumugam, M.K., Paal, M.C., Donohue, T.M., Ganesan, M., Osna, N.A. and Kharbanda, K.K. (2021) Beneficial Effects of Betaine: A Comprehensive Review. Biology, 10, 1-24. https://doi.org/10.3390/biology10060456

[19]. Uthpala, T.G.G., Marapana, R.A.U.J., Lakmini, K.P.C. and Wettimuny, D.C. (2020) Nutritional Bioactive Compounds and Health Benefits of Fresh and Processed Cucumber (Cucumis Sativus L.). Sumerianz journal of technology, 3, 75-82. https://doi.org/10.13140/RG.2.2.17510.04161

[20]. Martin, E., Anson, Zhang, and Campiche, R. (2022) Saccharide isomerate ameliorates cosmetic scalp conditions in a Chinese study population. Journal of Cosmetic Dermatology, 22, 262-266. https://doi.org/10.1111/jocd.14913.

[21]. Metin, Z.G., Helvaci, A. and Eren, M.G. (2021) Effects of Aloe vera in adults with mucocutaneous problems: A systematic review and meta-analysis. Journal of Advanced Nursing, 77, 1–22. https://doi.org/10.1111/jan.14653

[22]. Rattanawiwatpong, P., Wanitphakdeedecha, R., Bumrungpert, A. and Maiprasert, M. (2020) Anti-aging and brightening effects of a topical treatment containing vitamin C, vitamin E, and raspberry leaf cell culture extract: A split-face, randomized controlled trial. Journal of Cosmetic Dermatology 19, 671–676. https://doi.org/10.1111/jocd.13305.

[23]. Schagen, S.K. (2017) Topical Peptide Treatments with Effective Anti-Aging Results. Cosmetics, 4, 1-14. https://doi.org/10.3390/cosmetics402001.

[24]. Telang, P. S. Vitamin C in dermatology, Indian Dermatology Online Journal, 2013, 4, 143-146. https://doi.org/10.4103/2229-5178.110593.

[25]. Widgerow, A.D., Ziegler, M.E., Garruto, J.A., Robinson, D.M., Palm, M.D., Janelle, H. and Bell, M. (2022) Designing topical hyaluronic acid technology-Size does matter. Journal of Cosmetic Dermatology, 21, 2865–2870. https://doi.org/10.1111/jocd.15027

[26]. Elie, M. and Kressaty, J. (2009) Anti-Wrinkle Hormone-type Cosmetic composition. Patent US-2009/0068219-A1.

[27]. Worrall, E. and Bortkun A. (2020) Cream for treatment of skin injured by the sun. Patent WO 2020/201377A1.

TABLE 1: ACTIVE COMPOSITIONS FOR EI PRO RETINOL SUNSCREEN (PRODUCT A), EI PRO RETINOL MOISTURIZER (PRODUCT B), EI PRO RETINOL ALPHA SERUM (PRODUCT C), EI PRO RETINOL BETA SERUM (PRODUCT D) & EI PRO RETINOL GAMMA SERUM (PRODUCT E).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1328-1343www.ijprajournal.comISSN: 2249-7781

Brand Name	Ingredient name	Alpha	Gamma	Beta	Moisturizer	Sunscreen
Rovisome Retinol moist(Retinol)	Aqua,Pentylene Glycol, Lecithin,Alcohol, Retinol; polysorbate 20; Potassium Phosphate	✔	✔	✔	✖	✖
Nanoactive Retinol	Retinol	✔	✔	✔	✖	✖
Granactive Retinoid	Dimethyl Isosorbide (and) Hydroxypinacolone Retinoate)	✖	✖	✔	✖	✖
Retinyl Palmitate	Retinyl Palmitate	✖	✔	✖	✖	✖
Ac vce	3-o-ethyl ascorbic acid	✔	✔	✔	✔	✔
Oligo Hyaluronic Acid	Oligo Sodium Hyaluronate	✔	✔	✔	✖	✖
Wk pep coll	Palmitoyl Tripeptide-5	✔	✔	✔	✔	✖
Niacinamide	Niacinamide	✔	✔	✔	✔	✖
Genencare® osmsba	Betaine	✔	✔	✔	✔	✖
Aloe Vera Extract	Aloe Barbadensis Leaf Extract	✔	✔	✔	✖	✖
Aquaxyl	Xylitylglucoside&Anhydroxylitol& Xylitol	✔	✔	✔	✖	✖

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Cucumber Extract	Cucumis sativus (cucumber) Fruit Extract.	✔□	✔□	✔□	✖□	✖□
Pentavitin	Saccharide Isomerate (and) Aqua (and) Citric acid (and) Sodium Citrate	✔□	✔□	✔□	✖□	✖□
Hydronov Pa	Aqua (and) Phenethyl Alcohol (and) Sodium Carrageenan (and) Sea Salt	✔□	✔□	✔□	✔□	✖□
RonacareBisabolol	Bisabolol	✔□	✔□	✔□	✖□	✖□
Vitamin E	Tocopheryl Acetate	✔□	✔□	✔□	✔□	✔□
Allantoin (Sallitoin)	Allantoin	✔□	✔□	✔□	✖□	✖□
Alpha Arbutin	Alpha Arbutin	✖□	✖□	✖□	✔□	✖□
Shea Butter	ButyrospermumParkii (shea) Butter	✖□	✖□	✖□	✔□	✖□

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Ds-ceramix	Ceramide np (and) Ceramide ap (and) Glycosphingolipids (and) Hydrogenated Lecithin (and) TetraacetylPhytosphingosine (and) Cholesterol (and) Stearic Acid (and) Water (and) Xanthan Gum (and) Caprylic/Capric Triglyceride (and) Glycerin (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) 1,2-Hexanediol	✖	✖	✖	✔	✖
5M Hyaskin	Sodium Hyaluronate	✖	✖	✖	✔	✔
Neuvachiol	Bakuchiol	✖	✖	✖	✔	✖
Renouvellance	Glycerin (and) Water (and) PorphyridiumCruentum Extract	✖	✖	✖	✔	✔
Suncat De	Water (and) Ethylhexyl Methoxycinnamate (and) Butyl Methoxydibenzoylmethane (and) Benzophenone-3 (and) Phospholipids (and) 1,3-Butylene Glycol	✖	✖	✖	✖	✔
Chem 1789	Avobenzone	✖	✖	✖	✖	✔
Carrot Seed Oil	Daucus Carota Sativa seed oil	✖	✖	✖	✖	✔

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TABLE 2: DETAILS ON PRIMARY SKIN IRRITANCY TEST PRODUCTS : EI PRO RETINOL SUNSCREEN (PRODUCT A), EI PRO RETINOL MOISTURIZER (PRODUCT B), EI PRO RETINOL ALPHA SERUM (PRODUCT C), EI PRO RETINOL BETA SERUM (PRODUCT D) &EL PRO RETINOL GAMMA SERUM (PRODUCT E), NEGATIVE CONTROL (0.9% ISOTONIC SALINE SOLUTION) AND POSITIVE CONTROL (1% W/W SLS SOLUTION)

Patch no.	Test products	Codes	Batch/ LT	Dosage form	Mfg. Date	Retest Date	Packaging	Application area	Capacity
1	EI PRO Retinol SUNSCREEN (BEIPRS(R)105221	A	BEIPR S(R)10522	Cream	13/05/2022	12/12/2022	Pump Bottle	Between scapula and waist	100g
2	EI PRO Retinol MOISTURIZER (BEIPRM(R)l05221	B	BEIPR M(R)lO 522	Cream	13/05/2022	12/12/2022	jar	Between scapula and waist	100 g
3	EI PRO Retinol ALPHA SERUM (BEIPRAS(R)10522l	C	BEIPR AS(R)l 0522	Serum	13/05/2022	12/12/2022	Pump Bottle	Between scapula and waist	50 g
4	EI PRO Retinol BETA SERUM (BEIPRBS(R)105221	D	BEIPR BS(R)l 0522	Serum	13/05/2022	12/12/2022	Pump Bottle	Between scapula and waist	50 g
5	EI PRO Retinol GAMMA SERUM (BEIPRGS(R)105221	E	BEIPR GS(R)l 0522	Serum	13/05/2022	12/12/2022	Pump Bottle	Between scapula and waist	50 g
6	Negative Control (0.9% Isotonic saline solution)	-	-	Solution	13/05/2022	12/12/2022	Pump Bottle	Between scapula and waist	100 g
7	Positive Control (1% w/w SLS)	-	-	Solution	13/05/2022	12/12/2022	Pump Bottle	Between scapula and waist	100 g

TABLE 3: SCORE FOR ERYTHEMA FOR (DRYNESS, WRINKLES) AND OEDEMA AS PER DRAIZE SCALE

Score for Erythema/ dryness/ wrinkles	Reaction	Score for Oedema	Reaction
0	No reaction	0	No reaction

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1	Very slight erythema/dryness with shiny appearance	1	Veryslight Oedema
2	Slight erythema/ dryness/wrinkles	2	Slight Oedema
3	Moderate erythema/ dryness/wrinkles	3	Moderate Oedema
4	Severe erythema/ wrinkles/scales	4	Severe Oedema

TABLE 4: THE MEAN SCORE OF ERYTHEMA AND OEDEMA AT T2 DAYS (24 HOURS AFTER PATCH REMOVAL)

Test material	**Total Score for Erythema**	**Total Score for Oedema**	**Total Score for Erythema +Oedema**	**Mean Score (irritation)**	**Conclusion on the Irritation Assessment**
EI Pro Retinol Sunscreen [BEIPRS(R)10522]	1.0	0.0	1.0	0.0	Non-irritant
EI Pro Retinol moisturizer [BEIPRM(R)10522]	1.0	0.0	1.0	0.0	Non-irritant
EI Pro Retinol Alpha Serum [BEIPRAS(R)10522]	0.0	0.0	0.0	0.0	Non-irritant
EI Pro Retinol Beta serum [BEIPRBS(R)10522]	0.0	0.0	0.0	0.0	Non-irritant
EI Pro Retinol Gamma Serum [BEIPRGS(R)10522]	0.0	0.0	0.0	0.0	Non-irritant

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Negative control(0.9% Isotonic saline solution)	0.0	0.0	0.0	0.0	-
Positive Control (1% w/w SLS)	43.3	8.0	51.0	2.1	-

FIGURE1: METABOLIC PATH IN EVALUATING RETINOID ACTIVITY AND TOLERABILITY.

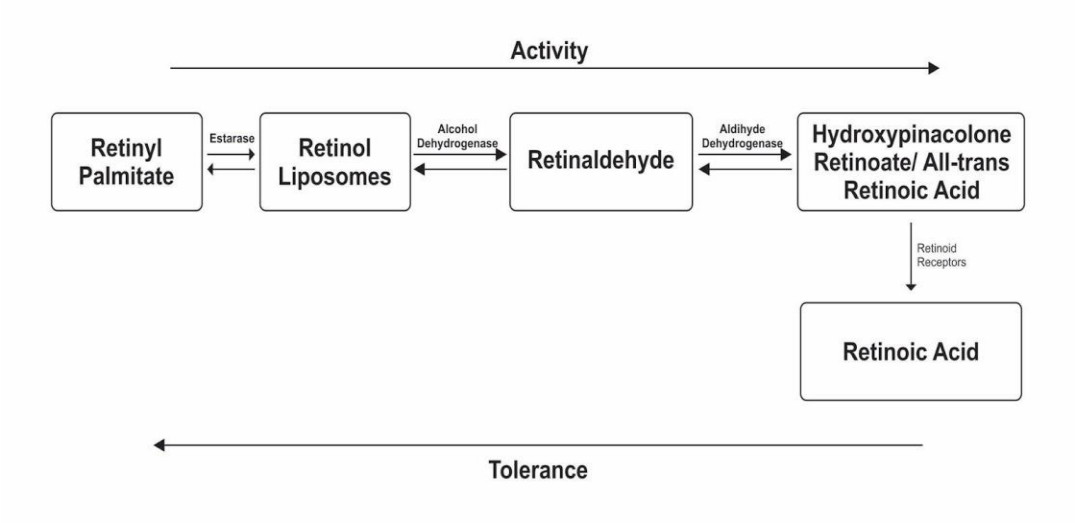

FIGURE 2: CONVERSIONS OF RETINYL ESTERS TO ALL TRANS RETINOIC ACID (ATRA) THROUGH DIFFERENT ENZYMES AND RETINOIC ACID RECEPTORS.

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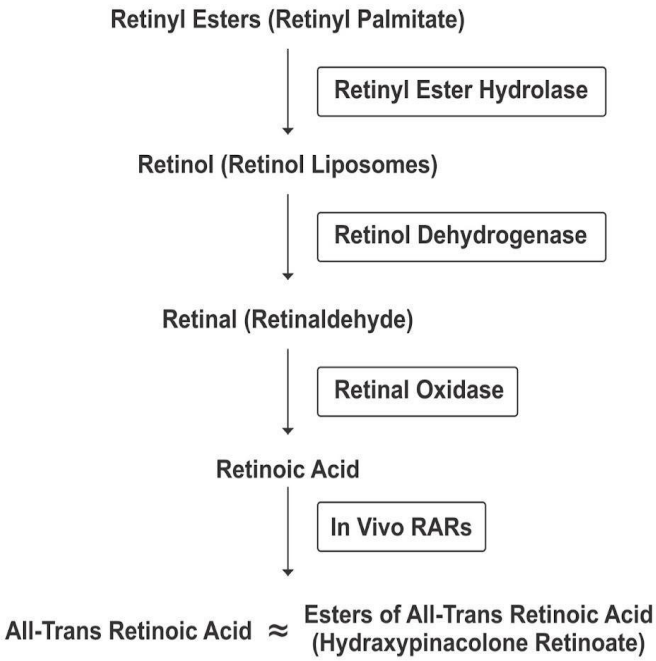

FIGURE 3: HERE ARE THE IMAGES OF EI PRO RETINOL ALPHA, BETA, GAMMA, MOISTURIZER, AND SUNSCREEN.

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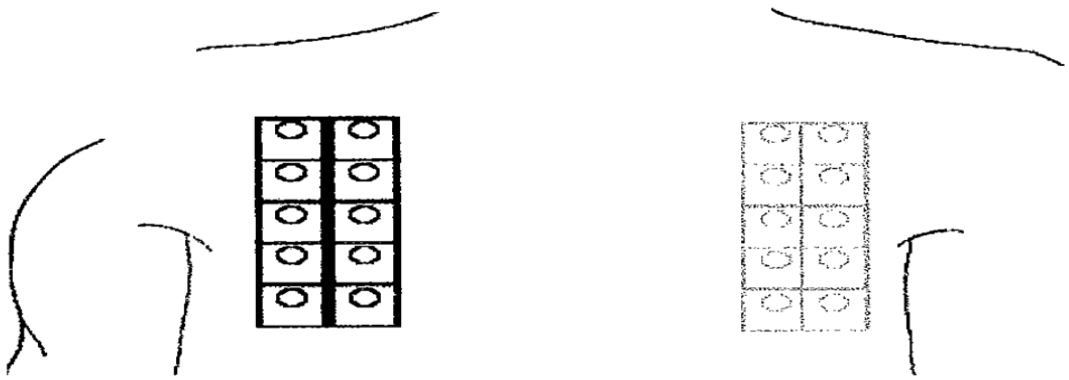
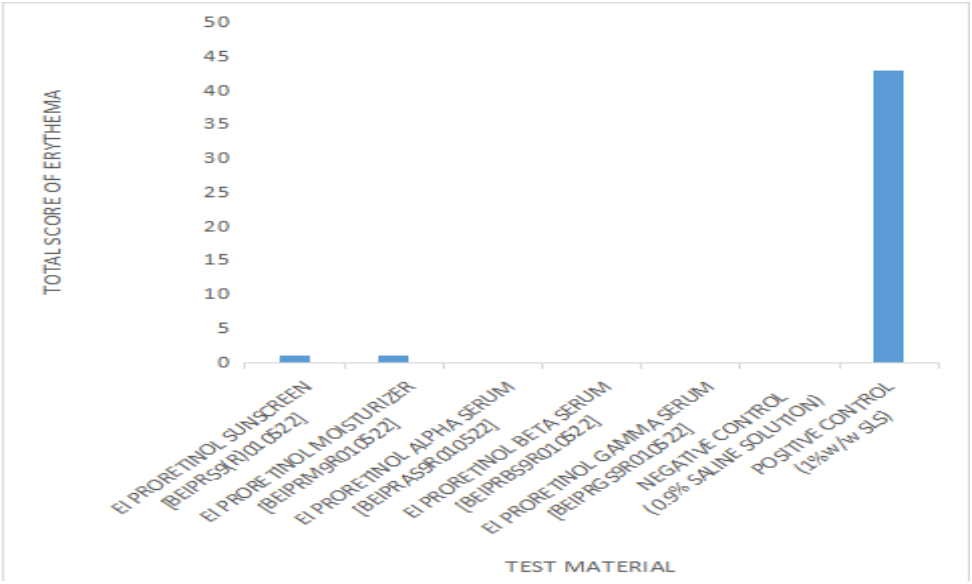
FIGURE 4: PATCH APPLICATION SITE BETWEEN SCAPULA AND WAIST ON THE BACK SHOULDER



GRAPH 1: TOTAL ERYTHEMA SCORE FOR EI PRO RETINOL SERIES, AGAINST NEGATIVE (0.9% ISOTONIC SALINE SOLUTION) , POSITIVE CONTROL (1% W/W SLS SOLUTION)



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GRAPH 2: TOTAL OEDEMA SCORE FOR EI PRO RETINOL SERIES, AGAINST NEGATIVE (0.9% ISOTONIC SALINE SOLUTION), POSITIVE CONTROL (1% W/W SLS SOLUTION)