

Evaluation of the efficacy and safety of “Anti-Wrinkle cream” in the treatment of facial skin wrinkles: A prospective, open, phase III clinical trial

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ABSTRACT

Aging of the skin is the result of continuous "wear and tear" processes. Chronological skin aging is a universal and inevitable process, while in contrast, photoaging results from the UV rays of sunlight, and the damage becomes apparent in sun-exposed skin. The “Anti-Wrinkle cream” is a polyherbal formulation recommended for the management of skin wrinkling and this study was planned to evaluate the efficacy and safety of the “Anti-Wrinkle cream” in the management of facial skin wrinkles.

ABBREVIATIONS

DNA : Deoxyribonucleic acid
LPS : Lipopolysaccharide
NO : Nitric oxide
PAF : Plasminogen activating factor
ROS : Reactive oxygen species
UVR : Ultraviolet rays

This study was a prospective, open, phase III clinical trial, conducted as per the ethical guidelines of Declaration of Helsinki. A total of 25 patients, of both sexes, aged from 35 to 65 years, with wrinkled facial skin, and who were willing to give informed consent were included in the study. Persons with known hypersensitivity to any of the ingredients of the formulation, any facial wound or abrasion, and who were not willing to give informed consent were excluded from the study. A baseline history was obtained in order to determine the patient’s eligibility for enrolment in the trial. All the patients were advised to apply the “Anti-Wrinkle cream”, twice-a-day for a period of 6 weeks. The subjective improvement evaluation was done by a predefined global grading system, which included the following gradations: “No improvement”, “fair improvement”, “remarkable improvement”, “very good improvement” and “excellent improvement”. All the patients were followed up for a period of 6 weeks, and at each weekly follow-up visit, the dermal safety and the improvement in skin wrinkling was evaluated. At the end of the 6th week, the overall performance of the “Anti-Wrinkle cream” was evaluated. The predefined primary end point was reduction in skin wrinkles, and the predefined secondary safety endpoints (for short- and long-term) were assessed by incidence of adverse events, and patient compliance to the therapy. All the adverse events reported or observed by the patients were recorded with information about the severity, date of onset, duration and action taken regarding the study drug. Statistical analysis was done according to intent-to-treat principles.

This study observed a significant improvement in the facial skin wrinkles, after a week’s application in almost all persons and also, there were no clinically significant adverse reactions, with excellent overall compliance. This beneficial effect might have been due to the synergistic antioxidant, anti-inflammatory, and UVR protective properties of the ingredients, which also have excellent safety profile. Therefore, it may be concluded that “Anti-Wrinkle cream” is effective and safe for usage in the management of facial skin wrinkles.

INTRODUCTION

Aging of the skin is the result of continuous "wear and tear" processes, which damage cellular DNA and proteins. Aging has been classified into 2 distinct types, viz. "chronological skin aging" and "photoaging" and both types have distinct clinical and histological features. Chronological skin aging is a universal and inevitable process, characterized primarily by physiologic alterations in skin function. In chronological skin aging, keratinocytes are terminally unable to form a functional stratum corneum, and the rate of formation of neutral lipids (which contribute to the barrier functions) gets slowed, resulting in dry, pale skin with fine wrinkles. In contrast, photoaging results from the UV rays of sunlight, and the damage becomes apparent in sun-exposed skin. Characteristics of photoaging are dry and sallow skin, displaying fine wrinkles as well as deep furrows, resulting from the disorganization of epidermal and dermal components associated with elastosis and heliodermatitis.¹

The "Anti-Wrinkle cream" is a polyherbal formulation recommended for the management of skin wrinkling, and it contains the extracts of *Aloe vera*, *Papaver rhoeas*, *Vitis vinifera*, *Citrus limon*, *Solanum lycopersicum*, *Santalum album*, *Rubia cordifolia*, *Saussurea lappa*, *Lens culinaris*, *Symplocos racemosa*, *Amomum subulatum*, *Curcuma longa*, *Glycyrrhiza glabra*, *Valeriana wallichii*, *Vetiveria zizanoides* and oil of *Triticum sativum*. This study was planned to evaluate the efficacy and safety of the "Anti-Wrinkle cream" in the management of facial skin wrinkles.

Aim of the study

This study was planned to evaluate the clinical efficacy and safety (short- and long-term) of the "Anti-Wrinkle cream" in the management of facial skin wrinkles.

MATERIALS AND METHODS

Study design

This study was a prospective, open, phase III clinical trial, conducted at the Department of Dermatology of Apollo Hospitals, Chennai, India, as per the ethical guidelines of Declaration of Helsinki, from June to September 2004. The study protocol, case report forms, regulatory clearance documents, product related information and informed consent form (in Tamil and English) were submitted to the "Institutional Ethics Committee" and were approved by the same.

Inclusion criteria

A total of 25 patients, of both sexes, aged from 35 to 65 years, with wrinkled facial skin, and who were willing to give informed consent were included in the study.

Exclusion criteria

Persons with known hypersensitivity to any of the ingredients of the formulation, any facial wound or abrasion, and who were not willing to give informed consent were excluded from the study.

Study procedure

A baseline history was obtained in order to determine the patient's eligibility for enrolment in the trial. The baseline assessment included personal data, a description of symptoms and details of past medical history (family history, history of possible exacerbating factor/s, etc.). All the patients were advised to apply the "Anti-Wrinkle cream", twice-a-day for a period of 6 weeks. The subjective improvement evaluation was done by a predefined global grading

system, which included following gradations: “No improvement”, “fair improvement”, “remarkable improvement”, “very good improvement” and “excellent improvement”.

Follow-up and monitoring

All the patients were followed up for a period of 6 weeks, and at each weekly follow-up visit, the dermal safety, and the improvement in skin wrinkling was evaluated. At the end of the 6th week, the overall performance of the “Anti-Wrinkle cream” was evaluated.

Primary and secondary end points

The predefined primary end point was reduction in skin wrinkles, and the predefined secondary safety endpoints (for short- and long-term) were assessed by the incidence of adverse events and patient compliance to the therapy.

Adverse events

All the adverse events either reported or observed by the patients were recorded with information about the severity, date of onset, duration and action taken regarding the study drug. Relation of adverse events to the study medication were predefined as “Unrelated” (a reaction that does not follow a reasonable temporal sequence from the time of administration of the drug), “Possible” (follows a known response pattern to the suspected drug, but could have been produced by the patient’s clinical state or other modes of therapy administered to the patient), and “Probable” (follows a known response pattern to the suspected drug that could not be reasonably explained by the known characteristics of the patient’s clinical state).

Patients were allowed to voluntarily withdraw from the study if they experienced serious discomfort during the study or sustained serious clinical events requiring specific treatment. For patients withdrawing from the study, efforts were made to ascertain the reason for dropout. Non-compliance was not regarded as treatment failure and reasons for non-compliance were noted.

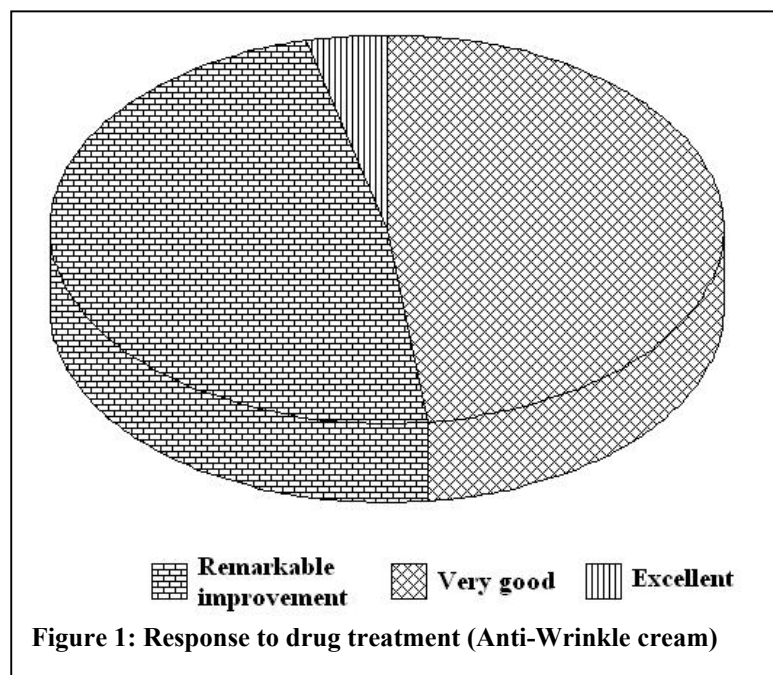
Statistical analysis

Statistical analysis was done according to intent-to-treat principles. The changes in various parameters from baseline values and the values after 6 weeks were evaluated by “Paired ‘t’ Test”. The minimum level of significance was fixed at 99% confidence limit and a 2-sided *p* value of <0.0001 was considered significant.

RESULTS

A total of 25 (15 females and 10 males) patients were enrolled in the study. There was significant improvement in

the facial skin wrinkles, after a week’s application, and the improvement trend continued, till the end of the study period. Out of 25 subjects, 12 (48%) had “remarkable” improvement, 12



(48%) had “very good” improvement, and 1 (4%) had “excellent” improvement in the facial skin wrinkles (Figure 1).

There were no clinically significant adverse reactions, either reported or observed, during the entire study period and the overall compliance to “Anti-Wrinkle cream” was excellent.

DISCUSSION

Complex biochemical processes precede aging, and it involves the ROS and free radicals. Results from various studies suggest that the antioxidative ability against ROS generation in the skin, possessed by antioxidant enzymes and low molecular weight antioxidants, is lowered age dependently.² A reduction in the number of epidermal Langerhans' cells is responsible for a decrease in delayed immune responsiveness in skin, as observed in the elderly, and reduced numbers of fibroblasts and mast cells are typical histologic findings in the aging human dermis. Collagen bundles also become fragmented, less elastic and more brittle. Telomere shortening at the end of chromosomes is probably the major mechanism of cellular senescence in skin.³

On the other hand that are the senescent keratinocytes become resistant to apoptosis and may survive for a long time (giving time for DNA and protein damage to accumulate with possible implication for carcinogenesis). The numbers of melanocytes decrease with age, and dysregulation of melanocyte density result in freckles, guttate hypo-melanosis, lentigines and nevi. The numbers of dendritic Langerhans cells also decrease with age and the cells get less dendrite, with reduction in the antigen-trapping capacity. Aging involves dermal changes, (such as damage to elastic and collagen fibers), giving thickened, tangled, and degraded non-functional fibers. Collagen intermolecular cross-links increase with age converting divalent cross-links into mature trivalent cross-links (of histidinohydroxylysinonorleucine)⁷ and the mechanisms involved are an enzyme-controlled process of maturation and a non-enzymatic glycosylation (the Maillard reaction), leading to cross-links in proteins (such as in collagen between arginine and lysine). Such kind of changes are also seen in case of patients with diabetes mellitus.⁴

Atmospheric pollutants are an important source of oxidative stress, and skin, which has a highly differentiated complex organizational structure, is particularly vulnerable to free radical damage because of its contact with oxygen and with other environmental stimuli.⁵

This study observed a significant improvement in the facial skin wrinkles, after a week’s application in almost all persons, and also, there were no clinically significant adverse reactions, with excellent overall compliance. This beneficial effect might have been due to the synergistic actions of the ingredients of “Anti-Wrinkle cream”, which have been documented by various researchers.

The principle constituents of *Aloe vera* are anthraquinones (aloe-emodin and aloin A/barbalin),⁶ Cinnamoyl, p-coumaroyl, feruloyl, caffeoyl aloesin, aloemannan⁷ acemannan, verectin⁸ elgonica dimer A and bisbenzopyran.⁷ *Aloe vera* has been long recognized as an effective remedy for wound-healing and other inflammatory skin disorders.⁹ Esteban et al. identified a peroxidase, which may scavenge H₂O₂ in skin surface and thereby prevent free radical damage of the skin.¹⁰ In another study, Byeon et al. demonstrated that *Aloe vera* contains multiple immunoprotective factors and oligosaccharides from *Aloe vera* prevent UVR-induced suppression of delayed type hypersensitivity (DTH), by reducing keratinocyte derived immunosuppressive cytokines.¹¹ In a study conducted by Heggors et al. treatment

with *Aloe vera* was found to expedite wound contraction, due to an increased collagen activity, consequently improving the collagen matrix and enhancing the breaking strength.¹²

The principle constituents of *Rubia cordifolia* are hydroxyanthraquinones, gallic acid, tannins¹³ alizarin, rubimallin, beta-sitosterol and daucosterol.¹⁴ Rubiadin, a dihydroxy anthraquinone, isolated from *Rubia cordifolia*, possesses potent antioxidant property, and prevents lipid peroxidation in a dose dependent manner.¹⁵ Tripathi et al. reported that, *Rubia cordifolia* maintained the reduced glutathione content in the physiological system.¹⁶

The principle constituents of *Vitis vinifera* are flavonoids (gallic acid, catechin, epicatechin, resveratrol, gallic acid, and ellagic acid)¹⁷ and glycosylated stilbenes: (Z)-piceatannol-(3,5,3',4'-tetrahydroxystilbene)-3-O-beta-d-glucopyranoside and (E)- and (Z)-resveratrol-(3,5, 4'-trihydroxystilbene)-4'-O-beta-d-glucopyranoside, which have potent antioxidant potential.¹⁸

The principle ingredients of *Citrus limon* are volatile oils and the major component is limonene.¹⁹ Naringin, a bioflavonoid predominant in *Citrus limon*, scavenges free radicals and reduces radiation-induced damage.²⁰ Calabrese et al. demonstrated the strong antioxidant activity of *Citrus limon* and reported a significant increase in the antioxidative potential of skin biosurface.⁵

The principle constituents of *Solanum lycopersicum* are lycopene, betacarotene, vitamin E,²¹ lutein²² and ferulic acid²³ all of which possess potent antioxidant potential. Balestrieri et al. observed that alpha-tocopherol in *Solanum lycopersicum* potentiates the effects of lycopene on the modulation of PAF and acyl-PAF biosynthesis in endothelial cells during oxidative stress.²⁴

The principle ingredient of *Santalum album* is santalol²⁵, santalbic acid²⁶ and alpha-transbergamotenol.²⁷ Jagetia et al. demonstrated the NO scavenging potential of *Santalum album*.²⁸

The principle constituents of *Saussurea lappa* are sesquiterpene lactones (costunolide and dehydrocostus lactone) and amino acid-sesquiterpene conjugates (saussureamines A and B), which inhibit NO production in LPS-activated peritoneal macrophages.²⁹ Jin et al. demonstrated the suppression of LPS-induced NO production by a dehydrocostus lactone of *Saussurea lappa*.³⁰ Cho et al. demonstrated that cynaropicrin, a sesquiterpene isolated from *Saussurea lappa* strongly inhibits TNF- α release from LPS-stimulated macrophage³¹

The principle constituents of *Symplocos racemosa* are salireposide and benzoylsalireposide.³² The active constituents of *Amomum subulatum* are protocatechualdehyde and protocatechuic acid, which have potent antioxidant activity.³³ Dhuley et al. documented that, *Amomum subulatum* supplementation increases the antioxidant enzyme activities, and the lipid conjugated dienes and hydroperoxides, which are the primary products of lipid peroxidation.³⁴

The principle ingredients of *Curcuma longa* are curcuminoids,³⁵ demethoxycurcumin, bisdemethoxycurcumin.³⁶ Alpha-curcumin, alpha-zingiberene, 1,8-cineole and zerumbone³⁷ Various studies have demonstrated the antioxidant potential of *Curcuma longa*.³⁸ The phenolic liposoluble 'co-antioxidants' of *Curcuma longa*, have a significant protective effect against age-related atherogenesis and immune dysfunction.³⁹ In a study by Ray et al., curcumin-treated cells showed a marked reduction in the expression of alpha-5-beta-1 and alpha-5-beta-3 integrin receptors. In addition, curcumin treatment inhibited pp125 focal adhesion kinase (FAK), tyrosine phosphorylation of a 120 kD protein, and collagenase

activity. Curcumin enhances the expression of antimetastatic proteins, tissue inhibitor metalloproteinase (TIMP)-2, nonmetastatic gene 23 (Nm23), and E-cadherin.⁴⁰

The active ingredients of *Glycyrrhiza glabra* are glabridin, glabrene, licochalcone A, licoricidin, licoisoflavone B, isoflavonoids (3-arylcoumarin, pterocarpan, and isoflavan), gancaonols, vestitol, licoricone, 1-methoxyphaseollidin, gancaonol C, glycyrin, formononetin, isolicoflavonol, glyasperin D, 6,8-diprenylorobol, gancaonin I, dihydrolicoisoflavone A, and gancaonol B,⁴¹ bioflavonoid licoagrodin, licoagrochalcones B, C, D, licoagroaurone, flavonoids (licochalcone C, kanzonol Y, glyinflanin B and glycyrdione A), isoflavone glycoside, licoagroside A, a maltol glycoside, licoagroside B⁴², licoleafol, uralstilbene, 8-dimethylallyleriodictyol, sophoraflavanone B, gancaonin R, and 6-dimethylallyleriodictyol⁴³, flavonoids (glabranin, pinocembrin, licoflavone, wighteone)⁴⁴ glabrizoflavone⁴⁵ isoliquiritigenin⁴⁶ and aglycone beta-glycyrrhetic acid.⁴⁷ *Glycyrrhiza glabra* has potent antioxidant activity.⁴⁸ In a study by Nose et al. the polysaccharide fractions of *Glycyrrhiza glabra* induced NO production by peritoneal macrophages.⁴⁹ In a study by Saeedi et al., *Glycyrrhiza glabra* has found to be an effective agent for the treatment of atopic dermatitis.⁵⁰

The principle ingredients of *Valeriana wallichii* are flavonoids (6-methylapigenin and hesperidin).⁵¹ The chemical components of *Vetiveria zizanioides* are sesquiterpenes (valencene, 9-octadecenamide, 2,6,10,15,19,23-hexamethyl-2, 6,10,14,18,22-tetracosahexaene, 1,2-benzendicarboxylic acid, and diisooctyl ester).⁵² Paranich et al. observed the tocopherol redox-system stimulation by *Triticum sativum*,⁵³ and Ferrando et al. reported the usefulness of *Triticum sativum* in diverse hyperkeratotic skin conditions (psoriasis, xerosis, pityriasis rubra pilaris, stuccokeratosis, seborrheic dermatitis, stasis dermatitis, and pityriasis lichenoides chronica).⁵⁴

The excellent effects of “Anti-Wrinkle cream”, might have been due to the synergistic antioxidant, anti-inflammatory, and UVR protective properties of the ingredients, which also have excellent safety profile.

CONCLUSION

Aging of the skin is the result of continuous "wear and tear" processes. Chronological skin aging is a universal and inevitable process, while in contrast, photoaging results from the UV rays of sunlight, and the damage becomes apparent in sun-exposed skin. The “Anti-Wrinkle cream” is a polyherbal formulation recommended for the management of skin wrinkling, and this study was planned to evaluate the efficacy and safety of the “Anti-Wrinkle cream” in the management of facial skin wrinkles.

This study observed a significant improvement in the facial skin wrinkles, after a week’s application in almost all persons; and also, there were no clinically significant adverse reactions, with excellent overall compliance. This beneficial effect might have been due to the synergistic antioxidant, anti-inflammatory, and UVR protective properties of the ingredients, which also have excellent safety profile. Therefore, it may be concluded that “Anti-Wrinkle cream” is effective and safe for usage, in the management of facial skin wrinkles.

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