



ORIGINAL ARTICLE

Pharmacological evaluation of anti-wrinkle creams containing estrogen vitamin C and E

Nayab Amber^{1*}, Ali Shareef² and Sajid Ali³

¹AH/CREATO writing and creative services Lahore, Pakistan

²Institute of Pharmacy, Lahore College for Women University, Lahore, Pakistan

³Department of Pharmacy, Forman Christian College University Lahore

Abstract

The skin is the largest organ in the body, accounting for around 15% of total body weight. The ageing process in humans is more evident in the skin in the form of fine lines and wrinkles. Aging can be brought on by a number of internal and external causes. Internal causes include the loss of estrogen hormone in postmenopausal women, which is one of the leading causes of skin wrinkle. To overcome above mentioned problems two topical creams were formulated, one containing estradiol and other one containing estradiol in combination with antioxidants i.e. vitamin C and E, which were evaluated physically. These were applied on the wrinkled skin of rats and their anti-wrinkle activity was observed. The skin of all three groups were examined for any reduction in wrinkles every three days for 15 days. At the day 0, there were about 15 to 20 wrinkles on 6 cm square area of rat's skin on average. But when the creams were applied and wrinkle scoring was observed every 3 days for 15 days the results of the creams containing estrogen and that containing estrogen + vitamin C & E were very good showing gradual decrease in wrinkles from 12, 15 on average to 2, 3 and even zero at the end of 15 days while the control cream failed to remove wrinkles from rats skin. Also the cream containing estrogen along with vitamin C and E showed the smoothness of skin and removes the dry patches and marks from the rat's skin. Efficacy of the preparations was determined by wrinkle scoring study of the skin of rats. The study showed the positive outcome of the experiments done on rat's wrinkled skin. It showed that estrogen cream should be used topically for the reduction of wrinkles and to improve the skin elasticity in advanced age population especially in post-menopausal women.

Keywords

Anti-wrinkle cream
Estrogen
Postmenopausal women
Vitamin C and E

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Introduction

Skin is the largest organ in the body, accounting for around 15% of an adult's total body weight. It covers the entire exterior structure of the body and acts as a protective shield for internal organs from the external environment, as well as controlling temperature and serving as an immunological barrier. The epidermis, dermis, and hypodermis are the three layers that make up the epidermis (Kolarsick *et al.*, 2011).

The skin is a preventive covering to the body, especially the stratum corneum, which is the outermost covering of the epidermis and prevents the body from deleterious climatic materials. It is also impermeable to loss of water. Skin also contains various glands such as eccrine glands, apocrine glands, sebaceous glands and melanocytes etc. (Lyons *et al.*, 2014).

In humans, the skin displays signs of ageing more than any other organ. Extrinsic (pollution, radiation, light exposure, chemicals) and intrinsic (hormone,

*Corresponding author: Email: doctornayab80@gmail.com

cellular metabolism, metabolic mechanism, genetics) factors combine to form a complex biological process. These factors combine to cause physiological and structural changes in the skin, as well as changes in its appearance (Ganceviciene *et al.*, 2012). With the increasing age, skin structure withers. A deprivation in elastin, collagen and intra-cutaneous fat occurs along with reduction in sweat and sebaceous glands with the progressing age (Lyons *et al.*, 2014). The development of wrinkles is the most visible symptom of skin ageing. Many histological studies on human facial skin indicate that as people get older, facial expression wrinkles become more permanent and well formed. Temporary wrinkles are created by smiling, frowning, or some other physical movement of the face as the skin loses its elasticity and thickness with age. In addition, the levels of hormones in the body change dramatically as people get older, especially estrogen levels in women (Takema & Nishijima, 1997).

Other factors responsible for wrinkle formation include sun light, cigarette smoking, and air pollution etc. Direct exposure to the sunlight damages the collagen which is the major protein of skin structure. Sunlight also destroys the elastin which causes the production of large quantities of enzymes named metalloproteinase's, which are responsible for damaging the collagen fibers and development of uneven skin. This abnormal skin is responsible for wrinkle formation. Oxidation is another important event in this whole process which occurs as a result of increase production of oxidants in the body. Oxidation also activates the metalloproteinase's (Simon *et al.*, 2000).

Estrogen is the primary sex hormone in women, and its levels in the body decrease as they get older. Estrogen has a major impact on the skin. Menopause's relative hypoestrogenism exacerbates the detrimental consequences of both extrinsic and intrinsic ageing, as shown by the skin's presence in the perimenopausal years. Estrogen enhances the appearance of the skin by increasing collagen content, thickness, and moisture. Estrogen receptors have also been found in skin in recent research (Verdier-Sévrain *et al.*, 2006). Various research comparing postmenopausal women taking estrogen replacement therapy to those who are not will help us better understand estrogen's impact in humans. The thickness of the epidermis rises in women who take oral estrogen compared to those who do not. When taken systemically or topically, hormone replacement therapy improves skin hydration. Since estrogen's molecular size is so tiny, it can easily penetrate the stratum corneum when applied topically. Furthermore, the levels of dermal hyaluronic acid and acid mucopolysaccharide rise, improving hydration (Wang *et al.*, 2007).

Vitamin C and E, on the other hand, are known for their antioxidant properties as well as their function in the collagen biosynthetic pathway (Humbert, 2003).

Various topical preparations containing vitamin C are present in the market. The most abundant anti-oxidant present in the human skin is vitamin C, which prevent the skin from reactive oxygen species (ROS). ROS are formed when epidermis comes in direct contact with UV light. Vitamin C acts by neutralizing the free radicals as it gives its electrons and prevents the skin from oxidation. Vitamin C works best in combination with vitamin E, as Vitamin C is hydrophilic and works in aqueous compartments of the cell, whereas, vitamin E being lipophilic in nature acts in the lipid portions of the cell so they both act synergistically (Telang, 2013).

As a result, we propose testing topical estrogen, as well as vitamin C and E, for anti-wrinkle activity in hairless albino mice. There is currently no topical preparation that contains estrogen as well as vitamins C and E.

A topical preparation can be developed in various forms such as creams, lotions, pastes, gels, ointments etc. Topical preparation is promptly spread on the skin and it has the benefit of ease of application and decrease side effects. These preparations usually contain active principle, drug and vehicles such as surfactant, absorbent, alcohol, water, oil, preservatives etc. various factors are involved in the penetration of the quantity of principle constituent via skin such as particle size of the chemicals, their penetration properties, thickness of the skin, amount of water in skin and preparation etc.

In topical preparation, we are proposing the production of estrogen cream along with vitamin C and E. Creams usually have moisturizing effect and also need the addition of preservatives. They usually comprise of emulsions of oil and water and are spread as a very thin layer on skin. Normally, 10 cm² area of skin is covered by the application of 1 gm of cream (Lyons *et al.*, 2014).

Materials and Methods

Experimental Animals: Albino rats which were 4 to 6 week old, weighing 50 to 75 gm were taken from animal house of Pharmacy, Physiology and Pharmacology Department of University of Agriculture, Faisalabad. Before the analysis, their hair was clipped and they were kept at room temperature for a week. They had unrestricted access to food and drink.

Development of wrinkles in mice: The hairless rats were divided into three groups each group containing 4 rats which were divided into 2 sub-groups. Each sub group contained one male and one female rats. To achieve the pronounced wrinkling, they were exposed to UVB irradiation for two weeks at a rate of five hours per day. Every day, before and after the application of the planning, photographs were taken (Duraivel *et al.*, 2014).

Preparation of cream containing estrogen: A water-in-oil emulsion was developed. 0.01% weight by weight

of 17-estradiol, phenolic anti-oxidants such as (butyl hydroquinone, butylhydroxytoluene, etc.), metal chelating agents such as EDTA, and one or more suspending agents e.g. (carboxymethylcellulose, methylcellulose), an emulsifying agent like (sodium lauryl sulphate), glyceryl monostearate, emulsifying wax, glycerol and purified water were used to prepare cream (Bequette *et al.*, 1984).

Preparation of cream containing estrogen with vitamin C and E: A water-in-oil emulsion was developed. 0.01% weight by weight of 17-estradiol, 1 to 10% weight by weight of vitamin C (ascorbic acid powder), 5% weight by weight of vitamin E (tocopherol), phenolic form anti-oxidants including (butyl hydroquinone, butylhydroxytoluene, etc.), metal chelating agents like EDTA, suspending agent e.g. (carboxymethylcellulose, methylcellulose), an emulsifying agent like (sodium lauryl sulphate), glyceryl monostearate, emulsifying wax, glycerol and purified water were used to prepare cream (Wilmott & Znaiden, 1991).

Preparation of control cream: Control cream does not contain any active ingredient. Oil in water emulsion was prepared and for this purpose two phases were prepared, oil phase and aqueous phase.

Determination of physical parameters of cream: The pH of prepared creams was determined using pH paper. Whereas, homogeneity and appearance of the cream was observed carefully every 24 hours for 15 days. The spread ability was determined by the time taken by two slides to slip off from the cream which has placed between those slides kept under certain weight. Time taken by slides for separation is inversely proportion to the spread ability.

Determination of acid value of creams: The acid value is the amount of potassium hydroxide required to neutralize one gram of the substance's free acid.

Saponification value: It's measured in mg of potassium hydroxide needed to saponify one (1) gram of fat. It is dependent on the type of fatty acid present in the fat.

Study of anti-wrinkle efficacy of creams in hairless mice: Mice were given the formulations once a day for 15 days, and wrinkles were measured by taking pictures every day and conducting skin wrinkle scoring analysis (Duraivel *et al.*, 2014). In control group, normal diet and light was provided and a control cream was applied for 15 days continuously on wrinkled skin. While, in group 1 estradiol cream was applied for 15 days and in group 2 estradiol + vitamin C & E cream was applied for 15 days continuously on wrinkled skin.

Results

Physical parameters of estradiol cream on room and accelerated temperature: The physical parameters of cream containing estrogen almost remained the same throughout the research at room and elevated

temperature. pH was fluctuated from 7 to 6.9. Homogeneity and spread ability of cream was good (Table 1). There was no change in color throughout the experiment. The type of smear remained mildly greasy with easy removal and cream had emollient effect.

Physical parameters of estradiol + Vitamin C & E cream on room and accelerated temperature: The physical parameters of all creams remain almost same but the color of cream containing estrogen in combination with vitamin C & E changed slightly at the tenth day due to the oxidation of ascorbic acid (Table 2). This change in color of cream can be overcome if we use L-methyl ascorbate instead of ascorbic acid in cream formulation.

Physical parameters of control cream on room and accelerated temperature: The physical parameters of control cream were also remained the same throughout the experiment at room and elevated temperature. There was not even any change in color of cream (Table 3). The pH fluctuated from 7.1 to 6.8.

Type of adverse effects of formulations: There was no adverse effects recorded for all three creams when they were applied on the rats' skin. All the creams did not show any sign of irritation, any kind of erythema or neither the edema on the skin of three groups of rats.

Acid and saponification values: The acid value for estradiol cream was 16.8 mg/KOH/0.5 gm while that of 20.2 and 35.8 mg/KOH/0.5 for estradiol + vitamin C & E and control cream respectively. However, saponification values for estradiol, estradiol + vitamin C & E and control cream were 191, 184.8 and 324.8 respectively.

Analysis of the visual evaluation of the reduction in the size of wrinkles in 6 cm square area after applying estradiol cream: At the day 0, there were about 13 to 15 wrinkles on 6 cm square area of rat's skin on average. But when the creams were applied and wrinkle scoring was observed every days for 15 days. At the day 3 there were about 10 to 13 wrinkles remained on the skin. At the day 6, the amount of wrinkles remained was 7 to 10 wrinkles on average. At day 9, wrinkle count was about 5 to 7. At day 12, they were reduced to about 2 to 3. At day 15, the wrinkle were completely removed from the skin of female rats while the male rat contains 1 wrinkle (Figure 1a). The results of cream containing estrogen + vitamin C & E were very good showing gradual decrease in wrinkles. At the day 0, there were about 16 to 18 wrinkles on 6 cm square area of rat's skin on average. But when the creams were applied and wrinkle scoring was observed every 3 days for 15 days. At the day 3 there were about 12 to 13 wrinkles remained on the skin. At the day 6, the amount of wrinkles remained 9 to 8 wrinkles on average. At day 9, wrinkle count was about 5 to 4. At day 12, they were reduced to about 2 to 1. At day 15, the wrinkles were completely removed from the skin of one female rat and one female rat contains 2 wrinkles while the wrinkles for one male rat were

Table 1: Physical parameters of estradiol cream on room and accelerated temperature.

| Formulation | Days | Temperature | pH | Homogeneity | Appearance | Spread ability | After feel | Type of smear | Removal |
|-----------------|------|-------------|-----|-------------|--------------------|----------------|------------|---------------|---------|
| Estradiol Cream | 0 | RT | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | 5 | RT | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 6.9 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | 10 | RT | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | 15 | RT | 6.7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |

Table 2: Physical parameters of estradiol + Vitamin C & E cream on room and accelerated temperature.

| Formulation | Days | Temperature | pH | Homogeneity | Appearance | Spread ability | After feel | Type of smear | Removal |
|----------------------------------|------|-------------|-----|-------------|------------------------|----------------|------------|-----------------|---------|
| Estradiol and Vitamin C+ E Cream | 0 | RT | 6 | Good | No change in color | Good | Emollient | Slightly greasy | Easy |
| | | 40°C ± 1°C | 5.9 | Good | No change in color | Good | Emollient | Slightly greasy | Easy |
| | 5 | RT | 6 | Good | No change in color | Good | Emollient | Slightly greasy | Easy |
| | | 40°C ± 1°C | 6 | Good | No change in color | Good | Emollient | Slightly greasy | Easy |
| | 10 | RT | 6.2 | Good | Slight change in color | Good | Emollient | Slightly greasy | Easy |
| | | 40°C ± 1°C | 6.1 | Good | Slight change in color | Good | Emollient | Slightly greasy | Easy |
| | 15 | RT | 6.2 | Good | Slight change in color | Good | Emollient | Slightly greasy | Easy |
| | | 40°C ± 1°C | 6.3 | Good | Slight change in color | Good | Emollient | Slightly greasy | Easy |

Table 3: Physical parameters of control cream on room and accelerated temperature.

| Formulation | Days | Temperature | pH | Homogeneity | Appearance | Spread ability | After feel | Type of smear | Removal |
|---------------|------|-------------|-----|-------------|--------------------|----------------|------------|---------------|---------|
| Control Cream | 0 | RT | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | 5 | RT | 6.9 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 6.8 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | 10 | RT | 7.1 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 7 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | 15 | RT | 6.8 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |
| | | 40°C ± 1°C | 6.8 | Good | No change in color | Good | Emollient | Mildly Greasy | Easy |

completely removed and 1 wrinkle was remained on one male rat. Also the cream containing estrogen along with vitamin C & E shows the smoothness of skin and removes the dry patches and marks from the rat's skin (Figure 1b). At day 0, there were about 15 to 17 wrinkles on the 6 cm square of rat's skin on average with control cream. But when the creams were applied and wrinkle scoring was observed every 3 days for 15 days. The control cream doesn't show any marked reduction in wrinkles. Only 4 to 5 wrinkles removed on average. At the 15th day after cream application there were almost 11 to 13 wrinkles which shows that control cream fails to remove wrinkles from the skin (Figure 1c).

Creams were applied on the wrinkled and marked skin of the rats for 15 days daily. Estradiol cream was applied on the wrinkled skin of group of rats marked with black marker (Figure 2a & b). Estradiol + vitamin C & E cream was applied on the wrinkled skin of group of rats marked with red marker (Figure 2c & d). Control cream

was applied on the wrinkled skin of group of rats marked with blue marker (Figure 2e & f).

Discussion

The skin is preventive covering to the body, specially the stratum corneum, which is outermost covering of the epidermis and prevents the body from deleterious climatic materials. It is also impermeable to loss of water. Skin also contains various glands such as eccrine glands, apocrine glands, sebaceous glands, melanocytes etc. (Lyons *et al.*, 2014).

In humans, the skin shows signs of ageing more than any other organ. Extrinsic (pollution, radiation, light exposure, chemicals) and intrinsic (hormone, cellular metabolism, metabolic mechanism, genetics) factors combine to form a complex biological process. These factors combine to induce physiological and structural changes in the skin, as well as changes in its appearance (Ganceviciene *et al.*, 2012).

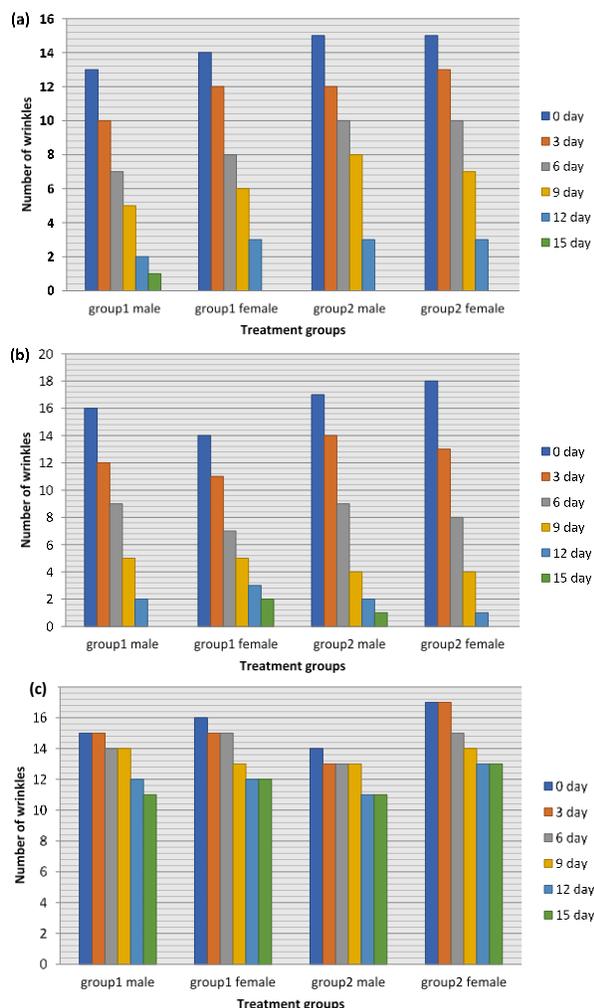


Figure 1: Wrinkle scoring analysis for estradiol cream (a), estradiol cream + vitamin C & E (b) and control cream (c).

The loss of estrogen after menopause has a significant impact on women's skin. The use of estrogen to treat postmenopausal women has been shown to increase collagen production, dermal thickness, elasticity, and skin water content. Furthermore, research on hormone replacement therapy (HRT) has shown that estrogen has an important effect on cutaneous injury repair (Anwar *et al.*, 2009).

Various research comparing postmenopausal women taking estrogen replacement therapy to those who have not will help us better understand estrogen's impact in humans. The thickness of the epidermis rises in women who take oral estrogen relative to those who do not. When used topically or systemically, hormone replacement therapy improves skin hydration. Since estrogen's molecular size is so tiny, it can easily penetrate the stratum corneum when applied topically. Furthermore, the levels of dermal hyaluronic acid and acid mucopolysaccharide rise, improving hydration (Wang *et al.*, 2007).

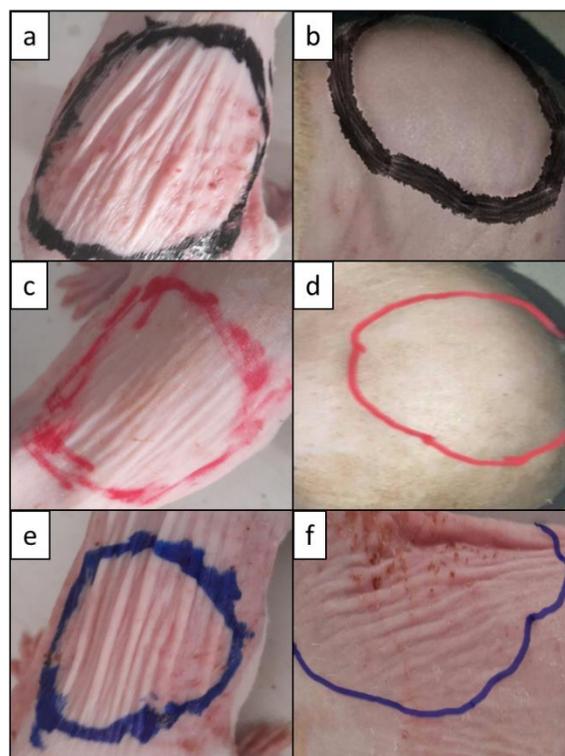


Figure 2. Visual evaluation of skin with wrinkles before application of cream (a, c and e), clear skin after the application of estradiol cream (b), clear skin after the application of estradiol + vitamin C & E cream (d) and skin after application of control cream (f).

Vitamins C and E are well-known for their antioxidant properties as well as their involvement in the collagen biosynthetic pathway (Humbert, 2003). Various topical preparations containing vitamin C are present in the market. The most abundant antioxidant present in the human skin is vitamin C which prevents the skin from reactive oxygen species (ROS). ROS are formed when epidermis comes in direct contact with UV light. Vitamin C acts by neutralizing the free radicals as it gives its electrons and prevents the skin from oxidation. Vitamin C works best in combination with vitamin E as Vitamin C is hydrophilic and works in aqueous compartments of the cell, whereas, vitamin E being lipophilic in nature acts in the lipid portions of the cell so they both act synergistically (Telang, 2013).

Two creams containing active ingredients were prepared and one cream which does not contain any active ingredient, it only contains base and referred as control cream. Creams containing active ingredients include estrogen cream and the cream which contain estrogen in combination with vitamin C and E. Their physical parameters, adverse effects on skin, acid value and saponification values were determined first. The physical parameters of all creams remain almost the same but the color of cream containing estrogen in

combination with vitamin C & E changed slightly at the tenth day due to the oxidation of ascorbic acid. This change in color of cream can be overcome if we use L-methyl ascorbate instead of ascorbic acid in cream formulation.

The hairless rats were divided into three groups each group containing 4 rats' two male and two female rats. To achieve the noticeable wrinkling, they were exposed to UVB irradiation for two weeks, for five hours per day. Every day, before and after the application of the cream, photographs were taken. Rat's wrinkled skin was marked with markers at 6 cm square area to for wrinkle scoring analysis (Manríquez *et al.*, 2014).

Creams were applied on the wrinkled and marked skin of the rats for 15 days daily. Control cream was applied on the wrinkled skin of group of rats marked with blue marker. Estradiol cream was applied on the wrinkled skin of group of rats marked with black marker. Estradiol + vitamin C & E cream was applied on the wrinkled skin of group of rats marked with red marker. Some procedures of application of cream and nano emulsion of *Moringa oleifera* seed oils was adapted by Duraivel *et al.* (2014).

The skin of all three rats groups was checked for any reduction in wrinkles every three days for 15 days. At the day 0, there were about 15 to 20 wrinkles on 6 cm square area of rat's skin on average. But when the creams were applied and wrinkle scoring was observed every 3 days for 15 days the results of creams containing estrogen and that containing estrogen + vitamin C & E were very good showing gradual decrease in wrinkles from 12, 15 on average to 2, 3 and even zero at the end of 15 days while the control cream fails to remove wrinkles from rats skin. Also the cream containing estrogen along with vitamin C & E shows the smoothness of skin and removes the dry patches and marks from the rat's skin.

Son *et al.* (2005) determined that when estrogen was applied topically on the skin of post-menopausal women and elderly males for two weeks, the thickness of epidermis increases. Our study also showed that when estrogen was applied topically on the wrinkled skin of rats for two weeks, the wrinkles were completely removed. Also, when the cream containing estrogen in combination with vitamin C & E was applied on the wrinkled skin, it also removed the dry patches along with the wrinkles. This combination cream also enhances the skin hydration and can be used to reduce freckles on skin.

Duraivel *et al.* (2014) prepared nano emulsion and oil in water cream from *Moringa oleifera* seed oil for the reduction of wrinkles on hairless mice. Both the preparations were applied on mice's wrinkled skin for 30 days. After 30 days, nano emulsion of *Moringa oleifera* seed oil show better results than the cream but after 30 days the mice's skin still has the wrinkles. While our preparations containing estrogen and that containing the

combination of estrogen with vitamin C & E were applied only for 15 days on the rat's skin and the wrinkles were completely removed from the skin. Same procedure of wrinkle development and application of preparation was adapted as that of Duraivel *et al.* (2014), but our results not only shows the removal of wrinkles but also increases the skin hydration along with the removal of dry patches.

Conclusion: The present study shows the usefulness of the experiments done on rat's wrinkled skin. It shows that estrogen cream can be used topically for the reduction of wrinkles and to improve the skin elasticity in aged population especially in post-menopausal women. It also shows that the estrogen can be used in combination with vitamin C and E which is the major antioxidants used in anti-aging products. The combination of estrogen with vitamin C & E can also be used to remove freckles and dry patches from the skin. After the successful animal trials human trials are needed for the further validation of this therapy. There is also the need of maintaining the doses of the cream formulations as estrogen also has the side effect of breast cancer but being topical formulation it has the advantage of less penetration to the systemic circulation and other body organs. It will have local effects mostly and also if we give this therapy to the post-menopausal women only than there will be no chance of any estrogen side effects because post-menopausal women are already deficient in estrogen and required estrogen to maintain various body functions. Also we have to work on the dosage intervals of the therapy. Further testing is required and this research can open new horizons in cosmeceutical industry.

References

- Anwar F, Hussain AI, Sherazi STH and Bhangar MI 2009. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (*Foeniculum vulgare* Mill.) fruit at different stages of maturity. *Journal of Herbs, Spices & Medicinal Plants*, **15**, 187-202.
- Bequette RJ, Hobbs LG and Scott JA 1984. Stabilized estradiol cream composition, Google Patents.
- Duraivel S, Shaheda A, Rabbani Basha S, Eesaf Pasha S and Jilani S 2014. Formulation and evaluation of anti-wrinkle activity of cream and nanoemulsion of moringaoleifera seed oil. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, **9**, 58-73.
- Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E and Zouboulis CC 2012. Skin anti-aging strategies. *Dermato-endocrinology*, **4**, 308-319.
- Humbert P 2003. Topical ascorbic acid on photoaged skin. Clinical, topographical. *Experimental Dermatology*, **12**, 237-244.

- Kolarsick PA, Kolarsick MA and Goodwin C 2011. Anatomy and physiology of the skin. *Journal of the Dermatology Nurses' Association*, **3**, 203-213.
- Lyons JJ, Sun G, Stone KD, Nelson C, Wisch L, O'Brien M, Jones N, Lindsley A, Komarow HD and Bai Y 2014. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. *Journal of Allergy and Clinical Immunology*, **133**, 1471-1474.
- Manríquez JJ, Cataldo K, Vera-Kellet C and Harz-Fresno I 2014. Wrinkles. *BMJ Clinical Evidence*, **2014**, 1-41.
- Simon H-U, Haj-Yehia A and Levi-Schaffer F 2000. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis*, **5**, 415-418.
- Son ED, Lee JY, Lee S, Kim MS, Lee BG, Chang IS and Chung JH 2005. Topical application of 17 β -estradiol increases extracellular matrix protein synthesis by stimulating TGF- β signaling in aged human skin in vivo. *Journal of Investigative Dermatology*, **124**, 1149-1161.
- Takema Y and Nishijima A 1997. Skin morphology at the time of UV irradiation is important. *Journal of the Society of Cosmetic Chemists*, **48**, 297-306.
- Telang PS 2013. Vitamin C in dermatology. *Indian Dermatology Online Journal*, **4**, 143.
- Verdier-Sévrain S, Bonté F and Gilchrist B 2006. Biology of estrogens in skin: implications for skin aging. *Experimental Dermatology*, **15**, 83-94.
- Wang F, Garza LA, Kang S, Varani J, Orringer JS, Fisher GJ and Voorhees JJ 2007. In vivo stimulation of de novo collagen production caused by cross-linked hyaluronic acid dermal filler injections in photodamaged human skin. *Archives of Dermatology*, **143**, 155-163.
- Wilmott JM and Znaiden AP 1991. Cosmetic preparation incorporating stabilized ascorbic acid, Google Patents.