

## HISTOLOGICAL EFFECTS OF ASCORBIC AND GLYCOLIC ACID COMBINATION ABSTRACT

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### ABSTRACT:

**AIM:** To observe the histological effects of combination of ascorbic and glycolic acid on skin.

**DESIGN:** Experimental randomized control trial study

**METHODS:** We selected ninety six female albino mice and randomly divided them into different experimental and control groups. The control group A received the application of aqua base cream and the control group B received nothing while the experimental group received the combination of ascorbic and glycolic acid. All agents were applied for six weeks duration after which no treatment was given for next six weeks to see whether the effects were temporary or permanent.

**RESULTS:** The microscopic changes were like increased number of layers in epidermis and increased thickness of dermis. We used paired t-test for the calculation of p-value.

**CONCLUSION:** The effects produced were temporary and reversed after stopping the treatment. The combination of ascorbic and glycolic acid is better choice for rejuvenation.

**KEY WORDS:** Combination of ascorbic and glycolic acid, epidermal thickness, reversible effects.

### INTRODUCTION:

The skin has very important cosmetic value for everyone and protects the underlying organs from the harmful effects of ultraviolet radiations. Aging produces the effects in all the organs of body. Skin being the most exposed part suffers a lot and depicts the effects of aging mostly as wrinkling and dryness<sup>[1,2]</sup>. Time and environment related factors produce aging in all the body organs but the exposed parts are more vulnerable. Especially the face shows the more pronounced age related changes<sup>[3,4,5]</sup>. The skin sagging, dryness and uneven pigmentation are the major signs of aging which are mainly due to decreased microcirculation with age<sup>[6,7,8]</sup>.

Day by day newer techniques are introduced for rejuvenation. As the exposed body parts like skin of face and hands are affected more so methods are also targeted to rejuvenate these organs. The surgical methods require proper skill and setups. Everybody wants to rejuvenate oneself with easily available method. The topical agents are a simpler and better choice as compared to other surgical procedures<sup>[9,10,11]</sup>.

Some fruit acids like glycolic and ascorbic acids are commonly used as rejuvenating agents for all types of skin. These are the superficial peels and easily available in the market and are quite economical. No expertise is required for their application. Their effects are localized as they only enter the superficial layers of skin<sup>[12,13]</sup>. These fruit acids improve the skin outlook by photo protection, acting as antioxidants, increasing the microcirculation and help in wound healing by stimulation of mitosis. These increase the dermal ridges so area of contact between epidermis and dermis is increased and stimulate angiogenesis leading to increased nutrients and oxygen supply to the epidermis. As the synthesis of collagen is dependent on ascorbic acid so increase the collagen bundles in the dermis<sup>[14,15]</sup>.

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Antioxidants are the agents which protect against the oxygen free radicals being continuously produced in the skin by exposure to ultraviolet radiations. The glycolic acid and ascorbic acid are the potent rejuvenating agents. These days they are commonly used in different skin care products in combination. So we studied their effects on skin in combination<sup>[8,16]</sup>.

## MATERIALS AND METHODS

**Study procedure:** This study was an experimental randomized controlled trial conducted at the Postgraduate Medical Institute (PGMI), Lahore. Ninety six female retired breeder Swiss albino mice were procured from the VRI, Lahore. Female mice of nine to twelve months of age weighing 25-30 grams were selected.

They were kept under controlled conditions<sup>1</sup>, in the experimental research laboratory of the Postgraduate Medical Institute (PGMI), Lahore.

They were provided standard mice diet and water ad libitum. After acclimatization of animals for one week, procedure was started. Swiss albino mice were randomly divided by balloting method into experimental and control groups with 32 mice in each. Each group was further subdivided into 1 & 2 consisting of sixteen mice. Subgroups 1 were sacrificed after six weeks and subgroups 2 were sacrificed after twelve weeks. Histological slides were prepared and examined under microscope.

## RESULTS:

All the data was collected, entered and analyzed by using the SPSS version 23.0. Epidermal and dermal thickness was measured in micrometers ( $\mu\text{m}$ ) presented by mean $\pm$ SD. These were compared by using paired t-test. Epidermal thickness comparison is shown by Table 2 while dermal thickness comparison is shown by Table 3. p-value<0.001 is taken as significant.

**Table 1**  
Groups Treated With rejuvenating agents

Groups		Duration Of therapy	Time at sacrifice	Treatment used
A	A1	6 Weeks	6 Weeks	Aqua base cream
	A2	6 Weeks	12 Weeks	
B	B1	-	6 Weeks	No treatment
	B2	-	12 Weeks	
C	C1	6 Weeks	6 Weeks	Ascorbic acid 15%+Glycolic acid10%
	C2	6 Weeks	12 Weeks	

**Table 2**  
Comparison of thickness of epidermis in  $\mu\text{m}$  in different experimental and control groups after 6& 12 weeks.

Groups	Mean $\pm$ SD		t-value	p-value
	6 week	12 week		
A	20.38 $\pm$ 1.13	19.56 $\pm$ 1.92	1.03	0.319
B	20.13 $\pm$ 2.05	19.13 $\pm$ 1.60	1.09	0.295
C	51.69 $\pm$ 5.41	22.31 $\pm$ 1.25	14.96	0.000*

\*p-value<0.001 significant

**Table.3**

Comparison of thickness of dermis in  $\mu\text{m}$  in different experimental and control groups after six and twelve weeks.

Groups	Mean $\pm$ SD		t-value	p-value
	6 week	12 week		
A	204.88 $\pm$ 11.64	202.00 $\pm$ 15.19	0.42	0.677
B	205.00 $\pm$ 17.38	200.81 $\pm$ 8.73	0.61	0.556
C	238.44 $\pm$ 15.86	208.75 $\pm$ 13.99	3.97	0.001*

\*p-value <0.001 significant

### DISCUSSION:

This experimental study was conducted to observe the effects of antiaging agents. In this study we observed the changes produced by the combination of different rejuvenating agents on cellular level in skin. We used the combination of topical rejuvenating agents like ascorbic acid and glycolic acid by using the experimental model of Swiss albino mice. According to our results use of combination rejuvenating agents is better choice and produced beneficial results<sup>[17]</sup>. All our results were for shorter period and did not produce any permanent changes at cellular level, so also favoring the safety of these agents. Any agent stimulating the mitosis for longer period of time may produce harmful effects<sup>[15,17]</sup>. Our study showed significant increase in the thickness of epidermis and dermis after application of topical treatment for six weeks duration in the experimental group as compared to the control groups. All changes produced were temporary and thickness was slowly reduced after twelve weeks of observation. Our results are comparable with the studies of Bhattacharya (2009). Other studies conducted by the scientists like Dhar (2009), Cho (2007) and Rachel (2003) also observed the similar results<sup>[1,3,17,18]</sup>.

Enhanced thickness of epidermis was the result of increased proliferation of basal cells by the effects of combination agents. Combination agents also stimulated the increased proliferation of fibroblast in the dermis resulting in increased thickness of dermis<sup>[18,19]</sup>. The combination of ascorbic and glycolic acid produced beneficial results in the experimental

group after six weeks as compared with control group by their topical application<sup>[20,21]</sup>. The superficial peel like glycolic acid helps in exfoliation of dead skin cells in superficial layers and stimulation of basal layer cells are very effective rejuvenating agents. The microscopic study of the skin samples correlated these results with the studies of Puizina et al., (2010); Rittie et al., (2008) and Rachel et al., (2003)<sup>[3,7,20]</sup>. The ascorbic acid is the major stimulus for production of collagen fibers in the dermis. The combination of these two agents is really helpful for rejuvenation in different skin compartments by proliferation of epidermal cells and enhanced synthesis of dermal collagen<sup>[3,17]</sup>.

We applied the agents topically as their local application is more beneficial for antiaging effects. The local contents of these agents increase many folds by their topical application as compared to their oral administration. So their topical use is more effective for skin rejuvenation<sup>[22,23]</sup>. These agents also act as antioxidants so, protecting the skin from harmful effects of ultraviolet radiations and free radicals. Now a day these are commonly used in different products like sun blocks, lotions and creams<sup>[14,24,25]</sup>. The antiaging effects of these agents are well established during their application period later on they are reduced. So the permanent skin changes are not produced by the application of these agents. The combination of different agents is better choice as combined effects of different agents are increased leading to enhanced response. Further researches are needed for production of better antiaging agent.

**CONCLUSION:**

Our study suggested that the combination of ascorbic and glycolic acid is better choice for skin rejuvenation. All the changes produced are for the treatment duration only and not permanent. This will help out for finding better antiaging agent.

**REFERENCES:**

1. Bhattacharyya, T.K., Higgins, N.P., Sebastian, J.S., Thomas, J.R., 2009. Comparison of epidermal morphological response to commercial antiwrinkle agents in the hairless mouse. *DermatolSurg*; 35(7):1109-1118.
2. Bhattacharyya, T. K. and Thomas, J. R., 2004. Histomorphologic Changes in Aging Skin Observations in the CBA Mouse Model. *Arch Facial PlastSurg*; 6 (1): 21-25
3. Rachel, J.D. and Jamora, J.J., 2003. Skin Rejuvenation Regimens, A profilometry and histopathological study. *Arch Facial PlastSurg*; 5(2):145-9.
4. Junqueira, L.C., Carneiro, J. 2010. Basic histology text and atlas. 12th ed. New York, McGraw Hill.
5. Fisher, G. J., Varani, J., Voorhees J. J., 2008, Looking Older, Fibroblast Collapse and Therapeutic Implications, *Arch Dermatol*; 144 (5):666-72.
6. Strandring, S., Collins, P., Wigley, C; 2008. Gray's Anatomy, The anatomical basis of clinical practice. 40th ed. London: Churchill Livingstone Elsevier.
7. Puizina-Ivic, N., Miric, L.C., Arija A., Karlica, D., Marasovi, D., 2010. Modern Approach to Topical Treatment of Aging Skin. *Coll. Antropol*; 34 (3): 1145-53.
8. Kafi, R., Kwak, H.S., Schumacher, W.E., Cho, S., Hanft, V.N., Hamilton, T.A., et al., 2007. Improvement of naturally aged skin with vitamin A (retinol). *Arch Dermatol.*;143(5):606-12.
9. Mukherjee, S., Date, A., Patravale, V., Korting, H.C., Roeder, A., Weindl, G., 2006. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clinical Interventions in Aging*; 1 (4): 327-348.
10. Baumann, L., 2007. Skin aging & its treatment. *Journal of Path*; 211(2):241-251.
11. Yaghoobi, R., Omidian, M., Bagherani, N., 2011. Comparison of therapeutic efficacy of topical corticosteroid and oral zinc sulfate-topical corticosteroid combination in the treatment of vitiligo patients: a clinical trial. *BMC Dermatol*;11:7.
12. Khunger, N., 2008. Standard guidelines of care for chemical peels. *Indian journal of dermatology, venereology, and leprology*;74: S5-S12.
13. Lugo, L.M., Lei, P., Andreadis, S.T., 2011. Vascularization of the Dermal Support Enhances Wound Re-Epithelialization by In Situ Delivery of Epidermal Keratinocytes. *Tissue Eng Part A*; 17(5-6): 665-675.
14. Loden, M., Buraczewska, I., Halvarsson, K., 2007. Facial anti-wrinkle cream: influence of product presentation on effectiveness: a randomized and controlled study. *Skin Research and Technology*; 13: 189-194.
15. Sauermann, K., Jaspers, S., Koop, U., Wenck, H., 2004. Topically applied vitamin C increases the density of dermal papillae in aged human skin. *BMC Dermatol*; 29: 4-13.
16. Kumari, R. and Thappa, D.M., 2010. Comparative study of trichloroacetic acid versus glycolic acid chemical peels in the treatment of melasma. *Indian J DermatolVenereolLeprol*;76(4):447.
17. Cho, H-S., Lee, M-H., Lee, J. W., No, K-O., Park, S-K., Lee, H-S., et al., 2007. Anti-wrinkling effects of the mixture of vitamin C, vitamin E, pycnogenol and evening primrose oil, and molecular mechanisms on hairless mouse skin caused by chronic ultraviolet B irradiation. *Photodermatology, Photoimmunology & Photomedicine*; 23(5): 155-162.
18. Dhar, H.L., 2009. Approach to anti-aging. *Bombay Hospital Journal*; 51(1): 68-72
19. Haftek, M., Mac-Mary, S., Bitoux, M-A. L., Creidi, P., Seit , S., Rougier, A., et al., 2008. Clinical, biometric and structural evaluation of the long-term effects of a topical treatment with ascorbic acid and madecassoside in photoaged human skin. *Experimental Dermatology*; 17(11): 946-952.

20. Rittié, L., Kang, S., Voorhees, J. J., Fisher, G. J., 2008. Induction of Collagen by Estradiol; Difference between Sun-Protected and Photodamaged Human Skin in Vivo. *Arch Dermatol*; 144(9):1129-1140.

21. Marcus, R., Coulston, A.M., 2006. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11<sup>th</sup> ed. New York, The McGraw Hill.




22. Decker, A. and Graber, E.M., 2012. Over-the-counter Acne Treatments. *The Journal of Clinical and Aesthetic Dermatology*; 5(5):32-40.

23. Helfrich, Y.R., Sachs, D.L., John, J.V., 2008. Overview of skin aging & Photoaging. *Dermatology Nursing*; 20 (3):177-183.

24. Puizina-Ivic, N., 2008. Skin aging. *Acta Dermatoven APA*. 17(2):47-54.

25. Watson, R.E.B., Ogden, S., Cotterell, L.F., Bowden, J.J., Bastrilles, J.Y., Long, S.P.,

Griffiths, C.E.M., 2009. A cosmetic 'anti-ageing' product improves photoaged skin: a double-blind, randomized controlled trial. *Br J Dermatol*; 161(2): 419-426.

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“PERFECT HAPPINESS COMES WITH KNOWLEDGE, AND PARTIAL HAPPINESS COMES WITH ABSTINENCE (CONTROL, FORBEARANCE, ZUHD), WORSHIP WITHOUT KNOWLEDGE AND WITHOUT ABSTINENCE MERELY EXHAUSTS THE BODY.”

**Hazrat Ali (Karmulha Wajhay)**