

# Comparative Study of Skin Rejuvenating Agents

RUKHSANA JABEEN<sup>1</sup>, NAZIA SIDDIQUE<sup>2</sup>, ZAIB<sup>3</sup>, AHMAD AMIN KHAN FARAZ<sup>4</sup>

## ABSTRACT

**Aim:** To compare the histological effects of different skin rejuvenating agents.

**Design:** Experimental randomized control trial study

**Methods:** Sixty four female Swiss albino mice were randomly divided into three experimental and two control groups. We applied vitamin C, hydroxyacetic acid and the combination of these two to the experimental groups. One of the control groups received vehicle and the other received nothing. All the agents were locally applied to the skin for six weeks duration after which treatment was stopped for next six weeks to see reversibility of the effects.

**Results:** The effects were like increased number of layers in epidermis and also increased thickness of dermis in all the experimental groups.

**Conclusion:** Combination rejuvenating agents were found better as compared to single agents and all the effects produced were temporary.

**Keywords:** Combination rejuvenating agents, epidermal and dermal thickness, reversible effects.

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## INTRODUCTION

Rejuvenation has been an issue of common interest since the start of this civilization. Everybody wants to rejuvenate oneself by using one or the other method<sup>1,2</sup>. The hall marks of skin aging are uneven discoloration, wrinkling and dryness. The skin microcirculation is badly affected by photoaging as well as environment related changes<sup>3,4</sup>.

The skin rejuvenation is quite interesting topic these days. Old age is now commonly understood as a disease that should be treated. These days the research work is mainly targeted in achieving the economical methods for rejuvenation<sup>6,7,8</sup>. Amongst the skin aging the facial skin, being the most exposed part to sunlight, is affected the most. Hence, the treatment options are also aimed to reverse the facial photoaging<sup>9</sup>. With the advent of newer techniques day by day the available treatment options are many. Each method has its beneficial effects over the other. But the choice of method varies with priorities of the patients<sup>10</sup>. Everybody wants to look younger, for which he uses the method which is easy as well as readily available. All the surgical methods require proper setup and expertise. So, the topically applied agents are more easily accessible and simpler choice<sup>11,12</sup>. Different chemical agents are being used as rejuvenating agents. The chemical agents like hydroxyacetic acid and vitamin C, which are also called fruit acids, are commonly being used for

rejuvenations. These penetrate the superficial layers of skin and local blood vessels. They produce localized effects in skin and improve its outlook. The oral administration of these agents does not enhance their local skin levels to such an extent to be comparable with their local absorption<sup>13,14</sup>.

These rejuvenating agents have multiple effects on skin to reverse the aging. They act as antioxidants, increase the microcirculation, help in healing of wounds and scars by stimulating the mitosis, increase production of collagen and create a photoprotective layer on the skin<sup>15,16</sup>. All these effects are responsible for increasing skin thickness. These also have effects on the dermal ridges and increase their number and height. So resulting in better transportation of nutrients from dermis to epidermis<sup>17,18</sup>.

## MATERIALS AND METHODS

This study was conducted at the Postgraduate Medical Institute (PGMI), Lahore. We obtained sixty four female retired breeders Swiss albino mice from the VRI, Lahore. All the animals were handled and kept properly according to the international and ethical guidelines for animal care. Swiss albino mice were randomly divided by balloting method into experimental and control groups with sixteen mice in each. Each group was further subdivided into 1 & 2 consisting of eight mice. Subgroups 1 were sacrificed after six weeks and subgroups 2 were sacrificed after twelve weeks. Histological slides were prepared and examined under microscope at different powers (Table 1).

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<sup>1</sup>Anatomy department, Sargodha Medical College, Sargodha.

<sup>2</sup>Associate Professor Anatomy, Lahore Medical & Dental College,

<sup>3</sup>Assistant Prof. Dermatology, Sargodha Medical college/ DHQ Hospital Sargodha.

<sup>4</sup>Amin Medical and Dental Care, Chiniot.

Correspondence to Dr. Rukhsana Jabeen Email: dr.rukhsanajabeen@yahoo.com

Table 1: Groups Treated With Rejuvenating Agents.

Groups	Duration of therapy (weeks)	Time at sacrifice (weeks)	Treatment used
<b>Group A</b>			Vitamin C
A1	6	6	
A2	6	12	
<b>Group B</b>			Hydroxy-acetic acid
B1	6	6	
B2	6	12	
<b>Group C</b>			Vitamin C + Hydroxyacetic acid
C1	6	6	
C2	6	12	
<b>Group D</b>			No treatment
D1	-	6	
D2	-	12	
<b>Group E</b>			Vehicle
E1	6	6	
E2	6	12	

**RESULTS**

All the data was collected, entered and analyzed by using the SPSS version 17.0. ANOVA (Analysis of Variance) post-hoc Tukey and paired t-test were used to calculate p-value. (Tables 2-5)

Table 2: Comparison of epidermal thickness amongst different groups after 6 weeks.

Comparison	Mean Difference	p-value (Tukey Test)
<b>Group A1</b>		
A1 VsB1	-4.00	0.139
A1 VsC1	-10.88	0.000*
A1 VsD1	20.69	0.000*
A1 VsE1	20.44	0.000*
<b>Group B1</b>		
B1 VsA1	4.00	0.139
B1 VsC1	-6.88	0.002*
B1 VsD1	24.69	0.000*
B1 VsE1	24.44	0.000*
<b>Group C1</b>		
C1 VsA1	10.88	0.000*
C1 VsB1	6.88	0.002*
C1 VsD1	31.56	0.000*
C1 V s E1	31.31	0.000*
<b>Group D1</b>		
D1 VsA1	-20.69	< .001*
D1 VsB1	-24.69	< 0.001*
D1 VsC1	-31.56	< 0.001*
D1 VsE1	-0.25	1.000
<b>Group E1</b>		
E1 VsA1	-20.44	< 0.001*
E1 VsB1	-24.44	< 0.001*
E1 VsC1	-31.31	< 0.001*
E1 VsD1	0.25	1.000

ANOVA post-hoc Tukey test was applied to calculate p-value. \*p- value< 0.05 significant

Table 3: Comparison of epidermal thickness amongst different groups after twelve weeks.

Comparison	Mean Difference	p-value (T test)
<b>Group A2</b>		
A2 VsB2	2.88	0.006*
A2 VsC2	1.63	0.238
A2 VsD2	4.81	0.000*
A2 VsE2	4.38	0.000*
<b>Group B2</b>		
B2VsA2	-2.88	0.006*
B2 VsC2	-1.25	0.493
B2 VsD2	1.94	0.110
B2 VsE2	1.50	0.312
<b>Group C2</b>		
C2 VsA2	-1.63	0.238
C2 VsB2	1.25	0.493
C2 VsD2	3.19	0.002*
C2 V s E2	2.75	0.009*
<b>Group D2</b>		
D2 VsA2	-4.81	0.000*
D2 VsB2	-1.94	0.110
D2 VsC2	-3.19	0.002*
D2 VsE2	-0.44	0.979
<b>Group E2</b>		
E2 VsB2	-4.38	0.000*
E2 VsC2	-1.50	0.312
E2 VsD2	-2.75	0.009*

ANOVA post-hoc Tukey test was applied to calculate p-value. \*p-value<0.05 significant

Table 4: Comparison of dermal thickness amongst different groups after six weeks.

Comparison	Mean Difference	p-value(T test)
<b>Group A1</b>		
A1 VsB1	6.19	0.920
A1 VsC1	-5.31	0.952
A1 VsD1	28.13	0.005*
A1 VsE1	28.25	0.005*
<b>Group B1</b>		
B1 VsA1	-6.19	0.920
B1 VsC1	-11.50	0.543
B1 VsD1	21.94	0.043*
B1 VsE1	22.06	0.041*
<b>Group C1</b>		
C1 VsA1	5.31	0.952
C1 VsB1	11.50	0.543
C1 VsD1	33.44	0.001*
C1 V s E1	33.56	0.001*
<b>Group D1</b>		
D1 VsA1	-28.13	0.005*
D1 VsB1	-21.94	0.043*
D1 VsC1	-33.44	0.001*
D1 VsE1	0.13	1.000
<b>Group E1</b>		
E1 VsA1	-28.25	0.005*
E1 VsB1	-22.06	0.041*
E1 VsC1	-33.56	0.001*
E1 VsD1	-0.13	1.000

ANOVA post-hoc Tukey test was applied to calculate p-value. \*p-value<0.05 significant

Table5: Comparison of dermal thickness amongst different groups after twelve weeks.

Comparison	Mean Difference	p-value (Tukey Test)
<b>Group A2</b>		
A2 VsB2	-2.19	0.998
A2 VsC2	-1.88	0.999
A2 VsD2	6.06	0.897
A2 VsE2	4.88	0.951
<b>Group B2</b>		
B2 VsA2	2.19	0.998
B2 VsC2	0.31	1.000
B2 VsD2	8.25	0.742
B2 VsE2	7.06	0.834
<b>Group C2</b>		
C2 VsA2	1.88	0.999
C2 VsB2	-0.31	1.000
C2 VsD2	7.94	0.768
C2 V s E2	6.75	0.856
<b>Group D2</b>		
D2 VsA2	-6.06	0.897
D2 VsB2	-8.25	0.742
D2VsC2	-7.94	0.768
D2 VsE2	-1.19	1.000
<b>Group E2</b>		
E2 VsA2	-4.88	0.951
E2 VsB2	-7.06	0.834
E2 VsC2	-6.75	0.856
E2 VsD2	1.19	1.000

ANOVA post-hoc Tukey test was applied to calculate p-value. p-value>0.05 non-significant

## DISCUSSION

The vitamin C is one of the commonest agents being component of a lot number of skin care and antiaging products. The hydroxyacetic acid is also a fruit acid and part and parcel of skin rejuvenating agents<sup>17,18</sup>. These agents are used singly as well as in combination. Our results showed better results with use of combination agents as compared to single agents. Hence the combination agents are better choice for skin rejuvenation with more beneficial effects<sup>17</sup>.

The changes produced were only for the period of treatment later on the effects diminished. So these skin rejuvenating agents do not produce permanent changes in the skin. Hence, these agents are quite safe for use for even prolonged periods without any side effects<sup>15</sup>. The microscopic changes showed significantly increased thickness of epidermis and dermis after the application of these topical rejuvenating agents in all the experimental groups as compared to control groups after six weeks. Many other scientists like Gulzar (2010), Dhar (2009) and Cho (2007) also found similar results<sup>6,17,19</sup>.

The combination of vitamin C and hydroxyacetic acid produced more profound results as compared to

their application as single agents. So their beneficial effects are multiplied when these are combined together. Epidermal thickness has increased as a result of increased cell division in the basal layers. Dermal thickness has increased as a result of increased proliferation of fibroblasts in the dermis<sup>18</sup>. All our experimental groups showed increase in epidermal and dermal thickness but the most significant results were obtained with the combination rejuvenation technique as compared to the controls as well as single agents<sup>19</sup>.

The vitamin C is the main factor responsible for hydroxylation process during collagen synthesis. It is also very helpful for wound healing and repair and increases the collagen bundles resulting in dermal thickness and skin rejuvenation<sup>20,21</sup>. The fruit acids like hydroxyacetic acid is also a powerful rejuvenating agent. It exfoliates the superficial epidermal layers thus removing dead cells and stimulate mitosis in basal layers. It rapidly renews the skin layers by speeding up their cell division. Our beneficial skin rejuvenation effects with combination agents are comparable with the results of Puizina et al., (2010); Rittie et al (2008) and Rachel et al (2003)<sup>3,7,21</sup>. The combination of vitamin C with hydroxyacetic acid combines the beneficial effects of these two resulting in enhanced effects as compared to the single agents<sup>22</sup>. In this experiment we applied all rejuvenating agents directly on the skin surface by which the local contents of these agents increased many folds. So their topical use is more effective for skin rejuvenation<sup>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</sup>. The combination of different agents is better choice as combined effects of different agents are multiplied leading to enhanced response<sup>25</sup>.

## CONCLUSION

Our study suggested that the combination of vitamin C and hydroxyacetic acid is better choice for skin rejuvenation as compared to single agent. These agents donot produce permanent changes in skin. This will help the customers for finding better rejuvenating agent.

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