POTENTIAL OF FLAVANOIDAL RICH HERBAL FORMULATION IN TREATING SKIN AGING COMPLICATIONS

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ABSTRACT:
Flavanoids are known to have efficiency on wrinkling, dry rough skin, red blotchiness and hyperpigmented spots in aged skin. Hyaluronidase and elastase are considered to be an important factor in degradation of Extracellular Matrix components (ECM) leading to bacterial invasion, envenomation of various toxins among others. This study potentiate that a flavanoidal rich component could safeguard the skin against the degradation caused by hyaluronidase and elastase and also from the generation of reactive oxygen species on UV exposure to UV Radiation. Most of the widely distributed medicinal plants, vegetables, fruit juices and a variety of beverages (tea, coffee, wines and fruit drinks) are found to have flavanoid as main component. Such herbs when incorporated into a suitable formulation exhibit a number of therapeutic actions at cellular level as well as at the Extracellular matrix (ECM) level. In order to protect the skin from harmful effects of UV radiation, matrix protein degrading enzymes like hyaluronidase and elastase are to be inhibited. The present communication reports the ability of a prepared flavanoidal rich herbal formulation in neutralizing the excessive ROS production but can also act as a promoter to collagen synthesis by inhibiting hyaluronidase and elastase activities and therefore, prolonging youthful skin.

KEY WORDS: Extracellular Matrix, Hyaluronidase, Elastase, Flavonoids.

INTRODUCTION:
The process of aging is a multistep event occurring in different level of the body. It can be designated as a cumulative destruction caused by the reactive oxygen species generation in response to the different pathological, chronological and environmental factors. UV radiation on long-term exposure to the skin causes acute and chronic effects in the human skin. The UV radiation causes generation of ROS and
reactive nitrogen species which ultimately leads to alteration in the structure and function of proteins which constitutes the extracellular matrix, for example collagen, elastin and glycosaminoglycans,[1]etc. The increased level of reactive oxygen species causes the formation of oxidative stress which further leads to DNA damage. All these consequences contributes in the development of skin related complications as the skin grows old like black patches, wrinkles and pits formation, dry and rough skin disorders etc.

Phytoactive drugs are being used from a long time to treat age related disorders. The main reason behind this approach is the synergistic approach which these herbs exist while treating any complications. Flavanoids serves to be an efficient agent which provide skin with desired strength and elasticity.[2] Current therapy of conventional drugs utilized for dermatological disorders are mainly based on topical preparations. These formulations relay on the passive diffusion of lipophilic and low molecular weight molecular components across the skin. In recent decades there has been a concerted effort to develop a novel and optimized formulation for enhancing topical effect. Stressed has been laid to develop such a herbal formulation that can treat all the associated disorders of the skin.[4-5] Hence, the objective of the present study is to formulate such a targeted formulation which can be helpful in treating various skin related complications manifested during the process of aging.

MATERIALS AND METHODS:

Quercetin pure isolated was procured from Sigma Aldrich, cetyl alcohol was procured from Himedia, glycerine (Merck), etc. Hyaluronidase type-1S from Bovine testes (999 units/mg solid) and Hyaluronic acid sodium salt from human umbilical cord, Elastase type IV from porcine pancreas, N-Succinyl-Ala-Ala-Ala-p nitroanilide (SANA) were purchased from Sigma Aldrich, USA. Dulbecco’s Modified Eagle’s Medium [Himedia ATO 68], Gentamycin [Nicholas Piramal]. All the chemicals used for the assay were of analytical grade (Merck and Qauligen).

Preparation of herbal cream:

All the ingredients were accurately weighed in order to formulate oil in water (O/W) cream by phase inversion method. The formulations were divided into two group (HC1, and HC2) One containing active drug herbal cream and another plain herbal cream. The solid phase was melted at 75°C. (Table No.1) To this the oil soluble were added and heated to 60°C. The pure isolated form of the drug was added [6-7]. When the temperature of both the phases reached 60°C. The aqueous phase was added gradually into oily phase with continuous stirring at 1500 rpm under magnetic stirring for homogenous mixing of the ingredients.

Determination of tyrosinase inhibitory activity

The method has been adoptated with slight modification from the tyrosinase inhibitory bioassay as discussed by Sharma et al. 2004 and Leu et al. 2008. In the present study we dissolved a significant amount of the herbal cream (10µL) in 0.1 ml of 10% dimethyl sulfoxide (DMSO) in an aqueous solution and incubated with 0.1 ml of L-tyrosinase (135 U/ml phosphate buffered solution [PBS], pH 6.8) at 37°C for 20 min. [7-8]To this 0.1 ml of 0.5 mM L-DOPA in a (PBS, pH 6.8) was added and further incubated the reaction mixture for 5 min. The amount of dopachrome in the mixture is determined by the optical density (OD) using microplate reader at 490 nm and the percent of tyrosinase activity inhibition was calculated according to given formula:
Inhibition (%) = \frac{(A-B) - (C-D)}{(A-B) \times 100}

Where A is the OD at 490 nm without herbal cream, B is the OD at 490 nm without the herbal cream but with tyrosinase, C is the OD at 475 nm with the herbal cream and D is the OD at 475 nm with the herbal cream, but without tyrosinase.

Determination of elastase inhibition

The above study was done with slight modification by Sahasrabudhe and Deodhar, 2010 in using Porcine pancreatic elastase which was assayed spectrophotometrically using N-Succ-(Ala) 3-nitroanilide (SANA) as the substrate and the release of p-nitroaniline was considered as a marker in estimating the degradation of ECM matrix by taking the absorbance for 15 min at 25°C at 410 nm. Different concentration of herbal cream (Ranging from 10-50µL ) was dissolved in Tris-HCl buffer with the reaction mixture containing 800 µl of 0.2 M Tris buffer (pH 8.0), 100 µl of enzyme elastase and 100 µl of 0.8 mM SANA as substrate. The herbal cream was preincubated with the enzyme for 20 min at 25°C. The reaction started when substrate was added to the reaction mixture. [9-12]As the reaction proceeds, the change in absorbance is monitored using UV spectrophotometer at 410 nm. Inhibitory effect of the samples on the Elastase activity calculated as:

\[
\text{Inhibition (\%)} = \frac{(A-B)}{AX \times 100}
\]

Where A, is the absorbance at 410 nm without test sample, and B is the change in absorbance at 410 nm with the test sample.

Determination of hyaluronidase inhibition

Hyaluronidases are considered to be the main ‘spliting factors’ and play a vital role in degradation of ECM leading to premature aging, dry skin syndrome, promote tumor growth and angiogenesis.[13]

The experiment was carried out according to the procedure described by Sahasrabudhe and Deodhar, 2010 with minor modifications. In the study hyaluronidase inhibition was determined by measuring the amount of N-acetylglucosamine splited from sodium hyaluronate. A concentration of 50 µL of herbal cream was dissolved in 5% DMSO and was mixed with 50µmL of hyaluronidase (7900 units ml−1) dissolved in 0.1 M acetate buffer having a of pH 3.6. The control was treated with 5% DMSO with the herbal cream and was incubated at 37°C for 20 minutes followed by a reincubation to the same stated condition with successive addition of 50µl of calcium chloride. This reaction mixture is further treated with 250 µl sodium hyaluronate and again incubated at 37°C for 40 min. [14-18] To the above reaction mixture, addition of 50 µl of 0.4 M sodium hydroxide and 100 µl of 0.2 M sodium borate was done and it was kept in the boiling water bath for 3 min and incubated. The reaction mixture was allowed to cool at room temperature and to this 1.5 ml of p-Dimethylaminobenzaldehyde solution was added. When this mixture is incubated for 20 min at 37°C, in a water bath, the colour change takes place which can be measured spectrophotometrically, at 585nm.[19]

Statistical analysis: The experimental results obtained were expressed as mean±standard error of mean (SEM). All measurements were carried out in triplicate.

RESULTS:

The result shows that the herbal cream formulated has the potential to treat the aging related complications. The HC1 when compared to HC2 taken as control, showed that the formulation significantly reduces the hyaluronidase activity, which is largely
responsible for the degradation of the extracellular matrix components. (Table. 2) The experiment showed that on increasing the drug concentration the desired effect can be achieved. Similarly the formulation was also found significant against inhibiting the elastase enzyme. The obtained value for tyrosinase activity inhibition is also encouraging. Both the activity vary significantly on varying the concentration of the drug sample.

DISCUSSION:

Quercetin is a naturally occurring flavanoid having higher impact on restoring the vitality of skin components as the drug encompass various inherent property in itself. The drug is antiproliferative, anticancer, antioxidant, etc. All these properties aids to redefine its role in preservation of skin in due course of aging. Elastin is an Extracellular Matrix protein which provides elasticity to the skin by causing the formation of elastic fiber in the dermis of the skin. In the due course of aging and due to environmental factors the skin loses its vitality due to deformation of the elastin protein. Damage to the elastin fibers causes decrease in skin flexibility. The skin contains enzyme called “proteinase” which is responsible for the degradation of the elastin. Therefore, to restore the suppleness and elegance of the skin elastase inhibition is of the major concerned to fight against skin aging complications. Similarly, Hyaluronic acid is key player in maintaining the luster of body. But it is short lived because of the activity of hyaluronidase enzyme. Hyaluronic acid keeps the body moist and that is the reason why dryness and roughness is remarkably noticed in aged skin. So, in order to maintain a balance between the body’s regulation in growing age it is very important to keep a check on excessive hyaluronidase enzyme activity. The tyrosinase is involved in the regulation of melanin pigment in the skin. Its excessive production causes darkening of the skin. Herbal drugs often possess cure to a series of disregularities occurring at different level because they have the unique nature to act synergistically. Quercetin, being an antioxidant can show its vitality at multiple steps. Its potentiality can be estimated by the above experiment which clearly shows that it can be prove beneficial in treating cosmetic complications in aging skin.

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Table: 1 Composition of Anti-aging herbal cream

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Ingredients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sesame oil</td>
<td>10%</td>
</tr>
<tr>
<td>2.</td>
<td>Tamarind gum</td>
<td>4%</td>
</tr>
<tr>
<td>3.</td>
<td>Isolated Quercetin</td>
<td>10%</td>
</tr>
<tr>
<td>4.</td>
<td>Cetyl alcohol</td>
<td>1%</td>
</tr>
<tr>
<td>5.</td>
<td>Glycerine</td>
<td>4%</td>
</tr>
<tr>
<td>6.</td>
<td>Rose oil</td>
<td>1%</td>
</tr>
<tr>
<td>7.</td>
<td>Water</td>
<td>70%</td>
</tr>
</tbody>
</table>

Table: 2 The effect of anti-aging herbal cream on hyaluronidase, elastase and tyrosinase Inhibition.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hyaluronidase Inhibition</th>
<th>Elastase Inhibition</th>
<th>Tyrosinase Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC1 (with drug)</td>
<td>52.32 ± 5.73</td>
<td>70.82±5.12</td>
<td>62.82±0.05</td>
</tr>
<tr>
<td>HC2 (without drug)</td>
<td>2.08±0.62</td>
<td>3.45±0.55</td>
<td>1.09±0.34</td>
</tr>
</tbody>
</table>

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