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RESEARCH ARTICLE

CELL CULTURE STUDY ON THE EFFECTS OF "CUREIT"- A NOVEL BIO AVAILABLE CURCUMIN ON HYALURONIDASE INHIBITION – ANTI AGING EFFECTS

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ABSTRACT

The hyalluronidases are a family of enzymes that degrade hyaluronic acid. Any ingredient that inhibits the hyaluronidases could serve as therapeutic targets in alleviating the age related functions. Curcumin was extensively used as an anti aging agent and in cosmetic creams. The main drawback of curcumin is its poor bio availability. This problem was encountered and made a novel bio available formulation known as "cureit". The "cureit" was checked for its potential to inhibit hyaluronidase, and found that it was inhibiting hyaluronidases up to 42% so that "cureit" could be a useful anti aging medication.

INTRODUCTION

Turmeric named by British as curry spice, is the dried rhizome powder of *Curcuma longa*, a perennial herb of the *Zingiberaceae* (ginger) family, which is 3–5 ft tall bearing oblong, pointed, short-stemmed leaves and funnel-shaped yellow flowers. The rhizome of turmeric is a valuable cash crop, which is widely cultivated in Asia, India, China, and other tropical countries. (Dobelis Hamper 1986) The major chemical principles of turmeric are curcuminoids, which impart characteristic yellow color to it. The curcuminoids can be separated from turmeric by ethanol extraction and it usually contains 0.3–5.4% curcumin (one of the major curcuminoids) depending on the season of its harvest (Srimal and Dhawan 1973). Human consumption of curcumin as a dietary spice ranges up to 100 mg/day (Jain and DeFilipps 1991) and recent phase I clinical trials indicate that humans can tolerate a dose of curcumin as high as 12 g/day, without any toxic side effects (Nadkarni 1954).

Curcumin and anti aging effects

Aging of the skin is a continuous process associated with increased wrinkles, deep lines and irregular pigmentation (Novoseltsev *et al.,* 2001). An important event in the process of aging is the production of reactive radical species by oxidative phosphorylation processes and from exogenous sources (Giacomoni and Rein G, 2001). Free Radicals are the cause of deterioration of the skin's supporting structures, leading to decreased elasticity and resilience (Giacomoni and Rein, 2001).

There are two primary skin ageing processes, intrinsic and extrinsic. Variations in individual genetic background are thought to govern intrinsic ageing, which results as time passes.(Chang HM and But PPH, 1986) By definition, this form of ageing is inevitable and, thus, apparently not subject to manipulation through changes in human behaviour. Conversely, extrinsic ageing is engendered by factors originating externally that are introduced to the human body, such as smoking, excessive alcohol consumption, poor nutrition, and chronic exposure to the sun. (Leung A, 1980) Exposure to such elements, which falls within the voluntary realm, although it may sometimes occur under duress, is not inevitable and thus represents premature skin ageing.(Chang HM and But PPH, 1986) Of these external factors, sun exposure is considered to be far and away the most significantly deleterious to the skin. Indeed, 80% of facial ageing is believed to be due to chronic sun exposure (Tu *et al.,* 1992).

In a study conducted in Thailand, curcuminoids loaded skin cream was found to be statistically significant in improving skin wrinkles, hydration, melanin content, biological elasticity, and viscoelasticity, compared with the cream base and the baseline from week 3 onward. The product showed

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no sign of skin irritation, both objectively and subjectively, throughout the study.

Hyaluronidase inhibition assay

Hyaluronic acid is a mucopolysaccharide, occurring naturally in all living organisms. This constitutes an important extracellular matrix in the various tissues like skin, lungs, ligaments etc. Some of the biological functions of HA include maintenance of the elastoviscosity of liquid connective tissues such as joint synovial and eye vitreous fluid, control of tissue hydration and water transport, supramolecular assembly of proteoglycans in the extracellular matrix, and numerous receptor-mediated roles in cell detachment, binds to water to lubricate the movable parts such as joints and muscles, mitosis, migration, tumor development and metastasis, and inflammation. HA is found primarily in the extracellular matrix and pericellular matrix, but has also been shown to occur intra- cellularly. HA also plays a major role in imparting volume to the dermis of skin by virtue of its affinity to water and hence its overall appearance. However during the process of aging and exposure to environmental insults, in the cellular systems in different organs and in the skin looses there arises an imbalance between the synthesis of HA and its respective degrading enzymes leading to reduced functionality. Inhibitors of hyaluronidase could serve as therapeutic targets in alleviating the age related compromised functions associated with imbalance of HA synthesis and hyaluronidases. Hyaluronidase inhibition is studied based on the spectrophotometric measurement of the chromophore reaction with para dimethyl amino benzaldehyde and is measured at 585nm.

Reagents and materials used

1. Test Compound – Curcumin
2. Bovine Hyaluronidase
3. 100mM Acetate Buffer (pH 3.5)
4. Sodium hydroxide
5. 0.2M sodium borate

MATERIALS AND METHODS

Hyaluronidase inhibition was determined by measuring the of N-acetylglucosamine splicing from sodium hyaluronate. Bovine hyaluronidase is used as a source of enzyme. 50 units of enzyme is dissolved in 100mM acetate buffer (pH 3.5) and incubated for 20 minutes with appropriate concentrations of the test sample. The enzyme reaction was initiated by addition of substrate and further incubated for 30 minutes. 50 µl of NaOH and 100 µl of 0.2 M sodium borate was added to the reaction mixture and then incubated in the boiling water bath for 3 minutes. After cooling to room temperature 1.5 ml of para dimethyl amino benzaldehyde was added and further incubated for 20 minutes at 37^{O}C. The developed colour is read at 585nm.

RESULTS AND DISCUSSION

The following is the result of the test performed

Hyaluronidase inhibitory property of bio available curcumin-"cureit" was studied in the concentration range of 10 to 150 µg/ml.

Table 1. Hyaluronidase Inhibition Activity – Consolidated Data

	% Inhibition				
Sample (µg/ml)	Batch I	Batch II	Batch III	Avg	SD
10	2.55	8.87	2.26	4.56	3.7
25	8.84	4.61	5.13	6.19	2.3
50	18.84	10.88	38.7	22.81	14.3
100	23.27	20.11	39.37	27.58	10.3
150	21.04	28.05	42.51	30.53	10.9

The "cureit" has shown a dose dependent inhibition with highest inhibition of 42% and reached saturation level around 200 µg/ml.

Conclusion

Hyaluronic acid is a mucopolysaccharide, occurring naturally in all living organisms. This constitutes an important extracellular matrix in the various tissues like skin, lungs, ligaments etc. Inhibitors of hyaluronidase could serve as therapeutic targets in alleviating the age related compromised functions associated with imbalance of HA synthesis and hyaluronidases. "cureit"- a novel bio available curcumin formulation was checked for its potential to inhibit hyaluronidase. The results of the study are given in Table1.As per the results, it was concluded that the bio available curcumin –"cureit" could be a useful anti aging medication since it has shown a highest inhibition of 42%.

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