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RESEARCH ARTICLE

GINGIVAL HERBAL DEPIGMENTATION: A NATURAL SOLUTION??

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Abstract

A smile not only enhances the beauty of an individual, it also boosts one's selfconfidence. Many factors such as lip position, tooth position, its morphology along with gingival tissue, attribute to the harmony of a beautiful smile. Gingival tissue health, color and contour are important components affecting an attractive smile. Amongst the gingival tissue, unsightly pigmented gingiva is of important concern for unpleasant smile. There are some prime pigments which contribute to the color of the gingiva. They are melanin, carotene, reduced hemoglobin and oxy-Hemoglobin. However, excessive melanin synthesis by active melanocytes (Unicellular dendritic cells) residing in the basal and suprabasal cell layers of the epithelium[1]cause its excessive deposition and unesthetic appearance of the gingiva. Various techniques have been developed to treat gingival hyperpigmentation[2,3,4.]However due to certain limitations of these techniques and due to growing demand for better aesthetics, many advances have been made to improve this untoward appearance. In this review we are planning to discuss and explore the herbal treatment modalities which can be appliedfor hyperpigmented gingiva with less potential of having side effects.

Introduction:-

C. P. Robinin 1873 first time used the term melanin and later melanocytes(the specialized cells responsible for melaninsynthesis) were identified. The melanin originates from the enzyme-catalyzed oxidation of phenolic and indolicsubstrates that polymerize and yield melanin. The melanin constitutes a diverse group of natural products found in most organisms, having functions related to protection against chemical and physical stresses. Thus, this melanindisplaysdistinct physicochemical properties. Their main biological functions include photoprotection[5] (it protects against UV induced DNA damage. It absorbs and scatters UV radiation. UV absorbed by melanin is converted into heat, a less toxic form of energy[6]) thermoregulation[7] free radical scavenging property[8]defense against predators and pathogens[9].Melanins have also been shown to haveantioxidant[10]and antiviral activities[11]. It also plays an important role in scavenging toxic drugs and chemicals.[12]

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Due to variety of reasons, there can be excessive production of melanin. It can be physiological or pathological. This condition is termed as hyperpigmentation. It can be seen in the skin as well as in the gingiva. It is known that the cellular structure of skin and the gingiva is the same[13]there are same melanocytes present in both the regions and also same melanin is produced. So, the same mechanism is responsible for hyperpigmentation in the skin as well as in the gingiva. It is justified to expect all depigmentation treatment modalities effective on skin to be beneficial for gingival hyperpigmentation. With this thought in mind this review article is presented.

Mechanism of Pigmentation (Melanogenesis):

In order to understand hyperpigmentation, we need to first understand the basic mechanism of melanin production in the body and the various enzymes which enable this process to occur. Melanin is produced in melanocytes in membrane bound organelles termedmelanosomesby a complex process called melanogenesis[14]Melanosomes have all the proteins (glycoproteinsPmel 17) and melanogenic enzymes necessary for melanin biosynthesis, for maintaining the structure of the melanosome, for maturation of immature pre-melanosome into a mature melanosome, and producing melanin.[15]Melanocytes interacts with endocrine, immune, inflammatory and central nervous system and as mentioned its activity is also regulated by extrinsic factors.Melanogenesis is a complex process which if disturbed, determines the different types of pigmentation defects which can be hypo or hyperpigmentation[16]Melanogenesis takes place by an increase in the intracellular cAMP concentration. The three core signalling pathways involved in the regulation of melanogenesis are: i) Melanocortin –1 receptor (MC1R) signalling, ii) The Wnt/β- catenin signalling pathway and iii) The tyrosine kinase receptor KIT/stem cell factor (SCF) pathway. All of which converge downstream to activate the master regulator (Microphthalmia associated transcription factor MITF). α-MSH binding to MC1R results in the activation of adenylyl cyclase, increasing the intracellular levels of cAMP and subsequently upregulating TYR, tyrosinase related protein-1 (TRP-1) and tyrosinase related protein-2 (TRP-2) expression. Thus, α-MSH-MC1R signalling pathway induces melanin production predominantly by elevating intracellular cAMP levels, thus its inhibition can exert inhibitory effects on melanogenesis. The Wntsignalling pathway has been previously reported to serve an important role in melanogenesis[17]Wnt ligands bind to Frizzled receptors on the cell surface, resulting in the increased stability of cytoplasmic β-catenin, and its subsequent translocation into the nucleus, where it activates the transcription of MITF. By regulating MITF transcription, the Wnt/β-catenin signalling pathway can control the expression of TYR and other pigmentation enzymes.MITF serves as the central hub of the regulatory network of melanin synthesis that is comprised of numerous transcription factors and signalling pathways that modulate the survival, proliferation and differentiation of melanoblasts and melanocytes. MITF serves an indispensable role in melanogenesis as it controls the transcription of TYR and other pigmentation-associated enzymes. Recent studies have also verified the important roles of the SCF- KIT signalling pathway in melanocyte proliferation and differentiation, and the process of melanogenesis**.** SCF is a paracrine factor that is secreted by fibroblasts, whereas c- KIT, its receptor, is expressed on melanocytes.[18]Thus, inhibitors of the SCF-KIT signalling pathway can potentially exhibit anti-melanogenesis activity.

In contrast to the regulation of gene expression of MITF by α-MSH and Wnt signal pathways, the extracellular signal-regulated kinase (ERK) pathway regulates melanogenesis via the degradation of the MITF protein[19]. Previous studies have shown that ERK activation phosphorylates MITF at serine 73, which is followed by MITF ubiquitination and proteasome-mediated degradation[20 21]. Therefore, activation of the ERK pathway would inhibit melanogenesis due to the down-regulation of the MITF activity. In addition, some reports have emphasized the important roles of c-Kit in the ERK pathway[22 23].

It is very important to note that the most important enzyme in the biosynthesis of melanin is tyrosinase. It is a copper containing, membrane bound, glycoprotein enzyme. It is a rate limiting enzyme and is critical for melanin synthesis. The synthesis and processing of tyrosinase enzyme happens in the Endoplasmic reticulum and the Golgi apparatus of the melanocytes present in the epithelium. Once it is synthesized, it is then transferred to specialized organelles called melanosomes. Here is where actual melanin synthesis occurs[24].The process starts with the oxidation of L-tyrosine to L-dopaquinone (DQ) in the presence of enzyme **Tyrosinase (TYR)**. Following DQ formation, the resulting quinone undergoes intramolecular cyclization and oxidation, where it serves as a substrate for the synthesis of eumelanin and pheomelanin. Hydroxylation of L-tyrosine to form L-3,4-dihydroxyphenylalanine (L-DOPA) is the rate-limiting step of the whole process.The melanosomes are transferred from the melanocytes to the surrounding keratinocytes[25]. Once in the keratinocytes, the pigment is distributed and thus imparts color.

Herbal extracts for lightening of hyperpigmented lesions

Traditional skin depigmenting agents such as Hydroquinone, corticosteroids are highly effective, however they raise several safety concerns such as allergic and contact dermatitis, melanocyte toxicity, atrophy, carcinogenesis[26]and other local and systemic side effects with long term exposure. Whereas, products of plant origin are gaining popularity as more and more individuals are inclined in adopting them in the field of cosmetics due to their herbal nature. Herbal products have been used since the ancient times and are known to be very much beneficial to health. They have less to no side effects and are non-toxic.[27] Many products derived from natural sources have been tried with great efficacy in treating hyperpigmented lesions of the skin[28] but fewer have been tried on the gingiva. An understanding of the benefits of natural and botanical extracts used on skin may provide an opportunity to develop new products to address gingival problems too.

The activity of tyrosinase enzyme needs to be targeted in order to developmodalities to treat or prevent hyperpigmented disorders.

Inhibition of tyrosinase enzyme can be brought about at different steps such as i) Modulation of catalytic activity ii) Transcription of messenger RNA iii) Maturation by glycosylation iv) Trafficking to melanosome.[29]

In order to develop modalities to treat or prevent hyper pigmentation, the catalytic activity of tyrosinase enzyme is targeted. This can be brought about by competitive or non- competitive inhibition of the catalytic activity of the enzyme.A competitive inhibitor is the one which bind to a free enzyme and prevents substrate binding to the enzyme active site.Since tyrosinase is a metalloenzyme containing copper,chelators of copper such as many aromatic acids, phenolic and poly-phenolic compounds, a few non-aromatic compounds, can inhibit tyrosinase competitively by mimicking the substrate of the enzyme[30 31].Non- competitive inhibitors bind to a free enzyme or to an enzyme–substrate complex with the same equilibrium constant[32].

The regulation of melanin synthesis is done by the enzymatic action of tyrosinase and thus by transcription of its encoding gene. Themaster regulator of melanogenesis-related gene expression is the Microphthalmia-associated transcription factor(**Tachibana et al., 1996**)[33]. It is a transcription factor with a basic-helix-loop helix-zipper motif. A number of factorswhich inhibit this transcription factor have been identified such as, ceramide (**Kim et al., 2002**)[34], Epigallocatechin-3-gallate and Hinokitiol **(Kimet al., 2004)[35].** These factors have been discussed further in detail.

Potential avenues include modulation of the transcription process of mRNA for tyrosinase and manipulation of the post transcriptional stability. Tyrosinase is also known to get degraded endogenously by proteosomes **(Halaben et al, 1997)[36].**However, these avenues are subjected to further research.Early studies show hydroquinone and azelaic acid to cause competitive inhibition of tyrosinase but were found to be cytotoxic to melanocytes. **(Jimbow et al,1974; Nazzaro- Porro and Passi, 1978)[37 38].** Whereasbotanical extracts arefound to be highly potent inhibitors of melanin formation and are not cytotoxic or mutagenic[39].

Various depigmenting botanical extracts:
The naturally occurring tyrosinase inhibitors are classified on the basis of their mechanism.

Inhibition of tyrosinase mRNA transcription

1. Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG), a major constituent of green tea, has been found to possess antioxidant, anti-inflammatory, and anti-carcinogenic properties (**Katiyar et al., 2001)[40].Kim et al, 2002[34],** conducted a study to check the effect of EGCG on melanin synthesis and tyrosinase in a spontaneously immortalized mouse melanocyte cell line, MeI-Ab. In particular, they checked for changes in MITF and tyrosinase protein production, and examined their effects on the ERK signalling pathway. Cells exposed to 10 μM EGCG showed decrease in pigmentation after 4 days when photographed under a phase contrast microscope. It was observed that EGCG treatment inhibited melanin synthesis in a concentration-dependent manner. Also, tyrosinase protein levels dropped after EGCG treatment in accord with the reduction in MITF protein level. This demonstrates that EGCG is a potent inhibitor of tyrosinase enzyme and hence caused a depigmenting effect.

2. Hinokitiol (ß- thujaplicin)

Hinokitiol was recently reported to decrease expression of tyrosinase and microphthalmia-associated transcription factor (MITF), which is a main transcription factor of the **TYR** gene, in murine melanoma cells(Kim et al,

2002).**Pillaiyar*etal*.(2017)**[41] reviewed on medicinal perspective of tyrosinase inhibitors, and discussed about the inhibitory potency and mechanisms of thujaplicin (α, β and γ isomers) as a human tyrosinase inhibitor. **Yoshimori*et al*., (2014)**[42] studied the inhibitory effects of three isomers of thujaplicin (α, β, and γ) on human tyrosinase and analysed their binding modes using homology model and docking studies, and found that β -thujaplicin (hinokitiol) to be a potent inhibitor of human tyrosinase. However, the mechanisms by which Hinokitiol decreases the intracellular levels of tyrosinase and MITF have not been fully elucidated. **Oyama et al, 2022**[43] investigated the underlying mechanisms using cultured human melanoma cells. Hinokitiolwas observed to decrease TYR protein level in a time- and dose-dependent manner in G361 human melanoma cells, while MITF protein level was decreased only at higher concentrations after 3 days treatment.

3. Ceramide

Ceramides are a common class of plant glycolipids which contain an amide-bound sphingoid base and no glycerol. Ceramides and other sphingolipids have biological activities, including anticancer properties, maintaining nerve function, and reducing cholesterol absorption **(Wehrmüller, 2007)**[44]**.** Ceramides can be extracted from rice, sweet potatoes, and yeast**. Kim et al, 2002**, studied the effects of sphingolipids on the growth and melanogenesis of human melanocytes. It was found that C2-ceramide inhibits cell growth in a dose-dependent manner. C2-ceramide also decreases the pigmentation of melanocytes by indirectly regulating tyrosinase, by decreasing the protein expression of microphthalmiaassociated transcription factor (MITF), which is required for tyrosinase expression. To identify the signalling pathway of ceramide, they studied the ability of C2-ceramide to influence extracellular signal-regulated protein kinase (ERK) and Akt/protein kinase B (PKB) activation. C2-ceramide was noted to induce a delayed activation of ERK (> 1 h) and a much later activation of Akt/PKB (>3 h) in human melanocytes. Hence it was concluded that, ceramide has potent depigmenting property.

I) Inhibition of tyrosinase enzyme activity

1. Arbutin (α Arbutin and Deoxyarbutin)

They are derivatives of arbutin, which is a naturally occurring beta-D-glucopyranoside derivative of hydroquinone. They are found usually in pear, cranberry, blueberry and bearberry shrub. Structurally they are simple phenols which are characterised by 1 aromatic ring and 1 or more hydroxyl group. They bring about inhibition of tyrosinase activity and DHICA polymerase activity atnoncytotoxic concentrations **(Maeda and Fukuda,1996; Chakraborty et al., 1998)**[45] [46]**.** It is thought that the activityof arbutin is driven by the structural homologies that it shareswith the substrate tyrosine, which leads to the competitiveinhibition of the catalytic function of tyrosinase. A study **(Funayama et al,1995)**[47] was conducted in which 100mg/g concentration of Deoxyarbutin was applied topically on hyperpigmented lesions on the skin for 15 days. It showed dramatic reduction in pigmentation. Hence, arbutin and its derivatives can be considered as a potential de pigmenting agent for oral hyper pigmentary lesions.

2. Aloesin

Aloe, a member of the Lily family, has been used for centuries in cosmetics. Aloesin is a naturalcompound isolated from aloe extracts. It has been shown in studies to modulate melanogenesis via competitive inhibition of tyrosinase. Aloesin inhibits purified tyrosinase enzyme and specifically inhibits melanin production in vitro. Tyrosine hydroxylase and DOPA (3,4-dihydroxyphenylalanine) oxidase activities (of tyrosinase from normal humanmelanocyte cell lysates) are inhibited by Aloesin in a dose dependant manner (**Jones et al., 2002**)[48]. **Choi et al, 2002**[49], conducted a study in which 100mg/g concentration of arbutin was applied 4 times a day for 15 days. Aloesin, along with arbutin, was observed to synergisticallyinhibit melanin production by combined mechanisms ofnon-competitive and competitive inhibitions of tyrosinaseactivity (**Jinet al., 1999**)[50].

3. Ellagic acid

Ellagic acid is polyphenol type of phytochemical. It is found in different kinds of fruits and vegetables like, strawberries, grapes, walnuts, blackberries, pecans etc. It is a widely studied phytochemical and is known to have anti-inflammatory and antioxidant properties. Along with this, ellagic acid has been studied to have anti melanogenic property as well. Animal studies showed that 1% ellagic acid effectively reduces hyperpigmented lesions, **(Shimogaki et al, 2000)**[51]. This depigmenting effect is attributed to its inhibitory effect on the tyrosinase enzyme. Ellagic acid inhibits tyrosinase enzyme by competing for the active binding site of tyrosine and L-DOPA by causing chelation of copper in tyrosinase enzyme. Hence, it can be considered as a potential depigmentation agent.

4. Licorice (*Glycyrrhiza glabra*)

The licorice root (*Glycyrrhiza glabra L.)* has long been employed in Western countries as a flavouring and sweetening agent, as well as a demulcent and expectorant. Many of licorice extracts such as glabridin and isoliquitrin showed significant antimicrobial activity in vitro [52 53]and antioxidant activity[54 55]. Glabridin has also been reported to inhibit the tyrosinase activity of melanocytes. It competitively inhibits the tyrosinase enzyme by chelating copper. Studies conducted on B 16 melanoma cells, demonstrated the effect of licorice extracts on melanin production. **Yokota et al**. [56] further showed that pigmentation and erythema induced by UV radiations, were inhibited by topical application of 0.5% glabridin. Liquiritin, another extract of licorice, causes dispersibility of melanin in melanocytes and thus leads to a depigmenting effect. **Amer and Metwalli et al, 2000**[57], demonstrated that 20% liquiritin in cream form applied at concentration of 1g/day for 4 weeks, significantly reduced melasma lesions of the skin. Notably, a drug delivery system of glabridinmicrosponge-loaded gel as a new approach for hyperpigmentation disorders has been proposed by **Deshmukh et al, 2012**[58].

5. Quercetin

Quercetin (3,3',4',5,7-pentahydroxyflavone), a flavonoid compound, is widely distributed in fruits, vegetables and tea, particularly in onions, black tea and apples (**Ravber et al., 2016**)[59]. It has many biological and pharmacological effects, including antioxidative, anticancer, anti-inflammatory and antiviral properties. (**Yang et al., 2016)**[60]**.** It was reported that quercetin can effectively inhibit tyrosinase activity by **Chen & Kubo, 2002**[61]. The inhibition is brought about by the oxidation of L-DOPA catalysed by mushroom tyrosinase andthis inhibitory activity comes from their copper chelating ability.**Jeong SH et al in 2009**[62]isolated 12 polyphenols possessing tyrosinase inhibitory properties from the methanol (95%) extract of Morus lhou. Of them quercetin was found to be a potent inhibitor of tyrosinase activity. It is brought about by competitive inhibition due to chelation of copper. **Meihui Fan et al (2017)**[63]conducted a study in which it was concluded that Quercetin could inhibit both monophenolase and diphenolase activities of tyrosinase. The binding of quercetin to tyrosinase was mainly due to their hydrophobic interactions which can induceconformational changes in tyrosinase. The catechol structure of quercetin is responsible for the chelation of the copper at the active site of tyrosinase which makes it a strong depigmenting agent.

6. P-coumaric acid

P-coumaric acid (*p*-CA), is a common secondary metabolite of plants. Coumaric acids are derivatives of cinnamic acid mono-hydroxylates at the phenyl group, and p-coumaric acid is the most abundant isoform. p-Coumaric acid is found at significant levels in many fruits, vegetables, and cereals. It has been observed to inhibit melanin formation in murine melanoma cells stimulated with α-melanocyte stimulating hormone (**An *et al.*, 2008**[64]**; Park *et al.*, 2008**[65]**)**. Due to its very close chemical structure to tyrosine, *p*-CA may interfere with the multiple pro melanogenic effects of tyrosine **(Slominski*et al.*, 1988**[66]**; Schwahn*et al.*, 2001**[67]). For example, *p*-CA may compete with tyrosine for active sites on TYR enzyme. In accordance with this notion, *p*-CA has been observed to inhibit the mushroom TYR-catalysed oxidation of tyrosine in a competitive manner **(Lim *et al.*, 1999**[68]**; Park *et al.*, 2008**). In another study[69]using human tyrosinase expressed in human embryonic kidney 293 cells, p-coumaric acid was shown to be the most potent inhibitor of human tyrosinase among the various phenolic acids tested. At a concentration of 3 µM, p-coumaric acid caused 50% inhibition of enzyme activity. A study conducted by **Sang Mi An et al (2010)**[70] compared the inhibitory effects of *p*-CA and two other well-known TYR inhibitors (arbutin and kojic acid) on the catalytic activities of mushroom, murine and human TYRs *in vitro*, using tyrosine and 3,4 dihydroxyphenylalanine (DOPA) as substrates. The results showed that *p*-CA is a strong inhibitor of human or murine tyrosinase in comparison with kojic acid and arbutin. In addition, *p*-CA inhibited human tyrosinase at much lower concentrations. Enzyme kinetic analysis indicated that *p*-CA is a competitive inhibitor (for DOPA) of human tyrosinase. Potent anti-melanogenic effects of *p*-CA were observed in human epidermal melanocytes exposed to UVB. Hence it can be concluded that *p*-CA is a potent and selective inhibitor of human TYR and is potentially useful as a hypo-pigmenting agent.

7. VitaminC (L- Ascorbic acid)

Vitamin C is a widely available substance found in a variety of plants, mainly in citrus fruits.L-Ascorbic acid (AA) functions in many biological processes, such as collagen synthesis, antioxidation, intestinal absorption of iron, and metabolism of some amino acids. AA is less resistant to oxidative conditions than other vitamins are and is easily decomposed, hence native ascorbic acid is modified to form different preparations such as, magnesium L-ascorbyl-2-phosphate and 2-O-a-D-glucopyranosyl-L-ascorbic acid.Vitamin C causes depigmentation in the skin by its inhibitory action on the enzyme tyrosinase. This is brought about the inhibition of the conversion of L-3,4-dihydroxyphenylalanine to L-3,4-dihydroxyphenylalanine quinone. The other non-enzymatic reactions in the

pathway of melanogenesis, are oxidative reactions hence antioxidants such as, magnesium L-ascorbyl-2-phosphate **(Kameyama et al.,1996)**[71] and 2-O-a-D-glucopyranosyl-L-ascorbic acid **(Kumano et al., 1998)**[72],were found to be effective inhibitors of melanin synthesis in cultured melanoma cells.**Kumano et al in 1998**demonstrated clinical improvement in melasma with 2% and 3% AA in cream form.**Kameyama et al., 1996** demonstrated a clinical improvement in an in vivo study inmelasma and senile freckles when a **10% topical formulation** of ascorbyl phosphate and magnesium was applied.Many studies have been conducted on animals[73] and human gingival tissues demonstrating a reduction in the melanin content and overall, a depigmenting effect.**Sheel et al (2015)**[74] carried out a depigmentation procedure in human gingival tissue, in vivo, by topical application of 10% of ascorbic acid. They demonstrated decreased melanin content and no recurrence even after 9 months of therapy. Hence ascorbic acid and it derivatives are proven to be potent and successful depigmenting agents on gingiva.

8. Resveratrol

Plants produce a large diverse class of polyphenols. One such polyphenol is stilbene. Resveratrol is the most common stilbene. Several stilbenes'derivatives from natural and synthetic sources havebeen investigated for their tyrosinase inhibition activity includingresveratrol from Morus alba[75]based on the enzymaticassays, resveratrol did not inhibit the diphenolase activity oftyrosinase, but L-tyrosine oxidation by tyrosinase was suppressedin presence of 100 mM resveratrol. Interestingly, after the 30 min ofpreincubation of tyrosinase and resveratrol, both monophenolaseand diphenolase activities of tyrosinase were significantlysuppressed. Furthermore, this effect was reduced with the additionof L-cysteine, which indicated suicide inhibition mechanismof resveratrol[76]. In addition to these studies on resveratrol, **Fachinettiet al 2018**[77]**,** have demonstrated that the incorporation of resveratrolinto nanostructured lipid carriers allowed tyrosinaseinhibitory activity which can contribute to a depigmenting effect.

Conclusion:-

Due to the vital role of tyrosinase in depigmentation disorders in humans, its inhibitors have been considered by researchers, extensively. Many plantsderived chemicals have wonderful potential as organic anti tyrosinase sources. Hyperpigmentation is an important issue and the most prominent target for inhibiting hyperpigmentation is tyrosinase, the rate-limiting enzyme in melanogenesis. Tyrosinase inhibitors have a huge demand in cosmetic and medicinal industries due to their preventive effect on pigmentation disorders as well as skin-whitening effect. These properties can be applied in the field of dentistry as well and products derived from these sources can be studied and used in the depigmentation of the gingiva.

Many herbal extracts have found to replace hydroquinone and other allopathic treatment modalities for pigmented lesions and are gaining popularity, whereas, out of these, only Vitamin C has been tried for treating pigmented lesions of the gingiva.This review aims to illustrate the various plant sources and their derived phytochemicals which can be potentially used as depigmenting agents and can create a new treatment modality for management of hyperpigmented gingiva.

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