



GLUTATHIONE AS A SKIN LIGHTNING AGENT IN COSMETICS

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ABSTRACT

Skin lighteners have been in use for ages in Asian countries where fairness is a major goal for every female and now it covered male section also. It is valued by western populations, who expose themselves excessively to the sun and develop sun spots on the skin as a consequence. Therefore this leads them to rely on different skin lighteners to achieve their goals and glutathione is one of them which help people to achieve skin lightening effect in a short period of time which gain popularity amongst celebrities and youtubers. Glutathione is a master antioxidant which is naturally present in human body and it is low molecular weight tripeptide that plays a prominent role in maintaining intracellular redox balance. Glutathione is present in its reduced form and plays an important role in various physiological functions. There is a skin lightening effects result from direct the inhibition of tyrosinase enzyme and switching from eumelanin to pheomelanin production. This article focuses on the various aspects of glutathione: its metabolism, mechanism of action, its side effects, and the scientific evidence to evaluate its efficacy as skin lightening agent.

KEYWORDS: Glutathione, skin lightening, tyrosinase activity, antioxidant.

INTRODUCTION

In India, many people are obsessed with fair skin and such peoples inspire others to desire fair complexion and sometimes seek it even against their will. Realizing the growing need for fair skin, many pharmaceutical companies are developing different molecules for skin lightning. The known depigmentary agent such as hydroquinone, glycolic acid, arbutin, kojic acid, vitamin C, vitamin E and niacinamide, all which are readily available. The advent of newer dipigmenting molecules such as Pycnogenol, Orchid and Marine Algae extracts, Cinnamic Acid, Soy, Aloesin and Boswellia has offered more topical options. The quest for systemic skin lightning logically ensued. Agents that have been promoted for this purpose include glutathione, tranexamic acid, vitamin C, different plant extracts and their combinations. Glutathione (GSH) is an antioxidant present in plants, animals, fungi, and some bacteria and the most important antioxidant found in mammalian cells. There are two source of Glutathione, i.e. natural source and synthetic source. Fresh fruits, vegetables, and nuts are natural sources of glutathione. Tomatoes, Avocados, Oranges, Walnuts and Asparagus are some of the common edibles that help to increase levels of glutathione in the body. Whey protein is another rich source of glutathione and has been used to enhance systemic glutathione levels in cystic fibrosis and L-glutathione, reduced glutathione, or GSH are synthetic glutathione dietary supplements. Glutathione was

discovered by J.de Rey-Paihade in 1888 from extracts of yeast and many animal tissues and in fresh egg white. J de-Rey-Paihade named this substance Philothione. In 1921, Hopkins suggested that the Philothion is a dipeptide consisting of cysteine and glutamate but these authors overlook the presence of glycine in Philothion possibly due to misinterpretation of the Van slyke amino N data. Honoring the history of the discovery of Philothion, Hopkins named the substance "glutathione" but in 1927 Hunter and Eagles indicated that glutathione is not a dipeptide but is a tripeptide consisting of Glutamate-cysteine and an additional low molecular weight amino acid. Using an acid hydrolysate of Glutathione, Hopkins proposed in 1929 that Glutathione is a tripeptide formed from cysteine, glutamate, and glycine. This proposal was supported by the independent work of Kendall and coworkers in 1929 and 1930. Based on titration of Glutathione in water and formaldehyde as well as the observed pK values, Pirie and Pinhey reported in 1929 that the structure of Glutathione is γ -Glutamate-Cysteine-Glycine. The structure of Glutathione was confirmed by Harington and Mead in 1935 through chemical synthesis from N-carbobenzoxycystine and glycine ethyl ester. One year later, another chemical synthesis of Glutathione was performed by du Vigneaud and Miller using S-benzylcysteinylglycine methyl ester and the acid chloride of N-carboarbobenzoxylglutamate- α -methyl ester.^[1, 2, 3] Glutathione is extensively used for producing

supplements and rising trend of supplement intake across the globe would increase a glutathione market share. Due to detoxifying and oxidative stress reducing properties these supplements have been found to be effective in treating health and skin issues and enable the human body to withstand foreign bodies. Glutathione has various functions, including DNA production, sperm cell formation, breaking down free radicals, regenerating vitamin E and C, assisting apoptosis (normal cell death), transporting mercury out of the brain, supporting immune function etc.^[4, 5] Deficiency of glutathione or decreased glutathione levels in the human body can lead to serious health issues therefore many celebrities and youngsters are using glutathione supplement for the healthier and fairer looking skin, they are also doing various advertisement on glutathione supplements and this leads to various media campaigns about glutathione and its exaggerated effects as a skin lightning agent. Over the counter availability of this drug have resulted in consumption of improper dose. Dermatologists who prescribe oral glutathione for general skin lightning or as an adjuvant for disorders of hyper pigmentation, are often unaware about its efficacy, dosing and adverse effects. This article highlights about its efficacy, its mechanism of action, pharmacokinetics and safety profile and check the current evidence supporting the efficacy of glutathione as a skin lightning agent.

MOLECULAR STRUCTURE

Glutathione is one of the most active antioxidant in human physiology and the structure of Glutathione was

confirmed by Harington and Mead in 1935. Glutathione is a small, low molecular weight, water soluble thiol-tripeptide formed by three amino acids (glutamate, cysteine and glycine) which was proposed by Hopkins in 1929.^[2,3] It has biologically active thiol group contributed by the cysteine moiety that acts as the active part of the molecule.^[4,5]

glutathione (GSH)

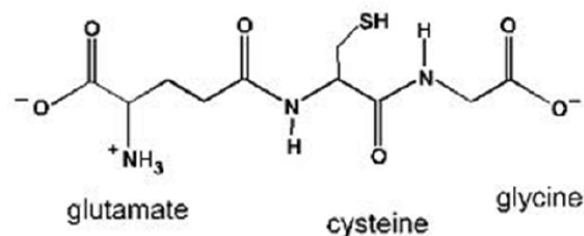


Figure 1: Molecular structure of glutathione.^[6]

Redox cycle

There are two interconvertible forms of Glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG). GSH is the predominant intracellular form, which acts as a strong antioxidant and defends against toxic compounds and xenobiotics. In this process, GSH is constantly oxidized to GSSG by the enzyme glutathione peroxidase. To maintain the intracellular redox balance, GSH is replenished through the reduction of GSSG by glutathione reductase enzyme.^[7]

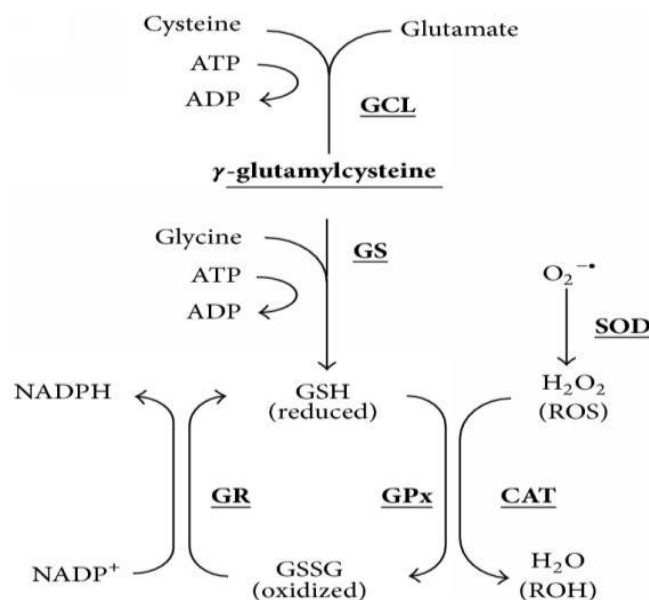


Figure 2: Redox cycle.^[8]

Functions of Glutathione

Glutathione plays a key role in multiple biological functions such as catalysis of exchange reactions, scavenging of free radicals, translocation of amino acids across cell membrane, Detoxification of xenobiotics, participation as a coenzyme in certain important

processes of cellular metabolism and maintenance of thiol groups of proteins and other molecules.^[5,6] Various researches have shown that many human diseases are associated with low level of glutathione. These include Emphysema, Asthma, Allergic disorder, Drug toxicity,

metabolic disorders, Cancer, Chemotherapy and Human immunodeficiency syndrome.^[9,10]

Routes of Administration

The three major routes of administration are topical, oral, and intravenous injections.

Topical Route

The role of glutathione as a skin-lightening agent was an accidental discovery when skin lightening was noticed as a side effect of large doses of glutathione.^[2] Glutathione can reduce tyrosinase activity in three different ways.^[11, 12] Tyrosinase is directly inhibited through chelation of the copper site by the thiol group. Secondly, glutathione interferes with the cellular transfer of tyrosinase to premelanosomes, a prerequisite for melanin synthesis.^[9] Thirdly, tyrosinase inhibition is effected indirectly via its antioxidant effect. Glutathione shifts melanogenesis from eumelanin to phaeomelanin synthesis by reactions between thiol groups and dopaquinone leading to the formation of sulfhydryl-dopa conjugates.^[13] Glutathione has potent antioxidant properties. The free radical scavenging effect of glutathione blocks the induction of tyrosinase activity caused by peroxides.^[13] Glutathione has been shown to scavenge ultraviolet radiation induced reactive oxygen species generated in epidermal cells.^[14] A recent study on melasma patients noted significantly higher levels of glutathione enzyme as compared to controls, confirming the role of oxidative stress in melasma.^[15] Based on these observations, the potential of glutathione in management of melasma and hyperpigmentation seems plausible.^[16] Glutathione is commercially available as face washes and creams.

Efficacy of Topical Route

A randomized, double-blind, placebo-controlled clinical trial conducted in 30 healthy Filipino women aged 30–50 years has provided some evidence favouring the efficacy of topical 2% GSSG lotion in temporary skin lightening. Patients were randomized to apply glutathione as 2% GSSG lotion and a placebo lotion in a split-face protocol, twice daily for ten weeks. GSSG was preferred over GSH, as GSH is unstable in aqueous solutions. GSSG eventually generates GSH after cutaneous absorption. The changes in the melanin index, moisture content of the stratum corneum, skin smoothness, skin elasticity and wrinkle formation were objectively assessed. The

reduction of the melanin index with glutathione was statistically significant when compared to placebo.^[17] Glutathione treated areas had significant improvement in other parameters as well. No adverse drug effects were reported. Glutathione has also become available in the form of soaps, face washes and creams.^[18] Recently, a glutathione based chemical peel has been launched. Although evidence of efficacy is lacking, the manufacturers claim improvement of melasma hyper pigmentation and skin ageing.^[19]

Oral Route

Oral glutathione is derived from *Torula yeast* (*candida utilis*). It is marketed as a food or dietary supplement, either alone or in combination with vitamin c, alpha lipoic acid and antioxidants. The principle site of absorption is the upper jejunum. Circulating glutathione is primarily cleared by the kidney.^[20] The bioavailability of oral glutathione in humans is a controversial subject. A single-dose study conducted by Witschi et al in seven healthy volunteers reported no significant increase in plasma glutathione levels for upto 270 min. However, Hagen and Jones reported an increase in plasma glutathione levels in four out of five subjects after a single oral dose of 15 mg/kg body weight. In that study, the plasma glutathione levels increased to 300% of baseline levels after one hour, followed by a decrease to approximately 200% of baseline levels within the next three hours.^[21,22] A randomized, double-blind, placebo-controlled study on oral glutathione supplementation(500 mg twice daily for four weeks)in 40 healthy adult volunteers failed to show any significant change in serum glutathione levels.^[23] Another randomized, double-blinded, placebo-controlled trial was conducted in 54 adults which administered Oral glutathione for six months, either in a dose of 250 mg or 1000 mg per day. Results showed a steady increase in glutathione levels when compared to the baseline. There are higher levels in the high dose group 30-35% increase vs. 17% increase in the low-dose group. The raised levels returned to baseline after a one-month washout period.^[24] In another study, glutathione administered at a single dose of 50 mg/kg body weight led to a considerable increase of protein-bound glutathione levels in plasma but not of the deproteinized fraction, measured after two hours of supplementation.^[25]

Table 1: Prescribed dosage of glutathione capsules/Tablets for skin lightening effects.

Dose	Time	Maintenance Dose
20-40 mg/kg body weight per day (i.e. 1-2 grams GSH per day) divided into two doses, for skin lightening effects.	Effects may become visible within four weeks; although a significant effect may need 1-3 months, 6-12 months and 2 years or more in brown skin, dark brown skin, very dark skin and black skin respectively.	After attaining the desired skin color, a maintenance dose of 500 mg/day for an indefinite duration has been suggested.

Efficacy of Oral Route

Randomized, double-blind, two-arm, placebo-controlled study conducted in the Thai population studied the effect

of orally administered glutathione on the skin melanin index in sixty healthy medical students. The subjects were randomized to receive glutathione capsules in a

dose of 500 mg/day in two divided doses, or placebo for four weeks. The primary end-point studied was the reduction of melanin indices at six different sites. At four weeks, the melanin indices decreased consistently at all six sites in the glutathione group. There was a statistically significant reduction at two sites in the placebo group, namely the right side of the face and the sun exposed left forearm. The tolerance to glutathione was excellent. The limitations of this study include a short study period, lack of follow-up, lack of measurement of serum glutathione levels and the choice of cohort, which consisted of a young and healthy population. Despite these shortcomings, this study was the first to demonstrate the beneficial effects of oral glutathione in skin lightening.^[26] Another open-label study that used glutathione containing lozenges reported improvement in the skin melanin index, as measured by Mexameter.^[27] They used buccal lozenges instead of capsules to enhance and ensure steady bioavailability. It is believed that the sublingual or buccal route is likely to increase the bioavailability of glutathione better than oral tablets or capsules.

Intravenous Route

Intravenous injections are being promoted to give therapeutic levels in the blood and skin and to produce instant skin lightening. Intravenous injections of glutathione have been used for years but there is not even a single clinical trial evaluating its efficacy. Manufacturers of intravenous injections recommend a dose of 600-1200 mg for skin lightening to be injected once or twice weekly. The duration for which they should be continued is not specified. Intravenous glutathione delivers a much higher therapeutic dose that enhances its efficacy; it also provides a narrower margin of safety due to the possibility of overdose toxic. In an animal based study, no significant adverse effects were reported in dogs, who were administered upto 300 mg of glutathione per kg body weight every day for 26 weeks.^[28] Human studies in which parenteral glutathione was administered for male infertility, or given to enhance insulin secretion in people with impaired glucose tolerance, did not report any significant adverse effects.^[29,30] However, the adverse effect of intravenous glutathione have been reported from the Philippines. The food and drug administration of Philippines have issued a position paper with a public warning regarding the safety of off-label use of glutathione injection and the adverse drug reactions reported from the use of intravenous glutathione for skin lightening.^[31]

Efficacy of Intravenous Route

A placebo controlled study was conducted on 50 patients, 25 patients in the group A and 25 patient in group B, aged 25-47 years. For every patient in group A, control of same skin tone and age was kept in group B, treated with placebo. Out of 50 enrolled patients 32 patients completed the study. In group A, 8 patients had deranged liver function and one patient who developed anaphylactic shock were excluded from the study along

with their controls from group B. 32 patients were treated in two groups. In group A 16 patients were given intravenous glutathione and vitamin C. patients in group B were given intravenous normal saline as placebo. Taylor hyperpigmentation scale was used to measure the skin tone. Two body sites, which were not exposed to sun, were measured with Taylor hyperpigmentation cards. Injection GSH Detox forte 1200 mg was given. Two injections per week for 6 weeks were given. The effectiveness and side effects were assessed at the end of therapy and 2 months and 6 months after cessation of treatment. After 12 injections of glutathione, 6 of 16 subjects showed significant improvement, whereas 3 subjects improved with placebo, after stopping the treatment, this improvement was gradually lost and at six month post treatment follow-up only one patient maintained this improvement. Adverse effects were noted in all patients such as abdominal cramps, deranged liver functions, and feeling of heart sinking, Diarrhea, paresthesia, Dizziness, Anaphylactic shock and vomiting.^[32]

Glutathione mesotherapy

Despite the lack of published literature on the efficacy and methodology of using glutathione solution as mesotherapy, it is widely practiced by dermatologists for the treatment of melasma and other facial melanoses. It is used as monotherapy, or in combination with ascorbic acid, vitamin E, tranexamic acid,^[33] etc. Although the results are claimed to be very good, use of glutathione as mesotherapy needs more evidence and published data.

ADVERSE EFFECT OF ORAL AND INTRAVENOUS ROUTE

Potential adverse effects of high dose and long term glutathione supplementation include:

- **Lightening of hair color**

A logically expected effect since hair color is dependent on the amount and type of melanin which may be altered by glutathione supplementation.^[34] This adverse effect has not yet been clinically reported.

- **Hypo pigmented patches**

Especially on sun-exposed areas have been observed after 10–12 doses of intravenous injection by practitioners (unpublished observations). Their experience suggested that the patchy hypo pigmentation tended to resolve after 30-40 doses due to the evolution of a uniform skin-lightening effect.^[34]

- **Depletion of natural hepatic stores of glutathione**

Hypothetically, long-term supplementation with any external synthetic compound may signal the body to stop its own production resulting in dependence on synthetic supplements. Depletion of liver glutathione levels (the site of glutathione storage) may be devastating to health. This hypothetical adverse effect, although not clinically reported until now, is analogous to the hypothalamic

pituitary axis suppression seen with long-term use of systemic corticosteroids.^[34]

- **Increased susceptibility to melanoma**

Theoretically, long-term administration of systemic glutathione switches eumelanin to pheomelanin, and may increase the development of melanoma in the long run.^[35]

- **Exacerbation of *Helicobacter pylori* associated peptic ulcers**

Helicobacter pylori are known to feed on macrophages and neutrophils abundant at the site of inflammation caused by the ulcer. As glutathione can improve the numbers and activity of macrophages, peptic ulcers may be exacerbated.^[36]

The above mentioned potential adverse effect are not scientifically proved yet but has the capacity to emerge in future as prominent adverse effects due to the increasing demand for glutathione supplementation and over the counter availability which tends to worsen the situation. So, further studies are needed to confirm their reliability.

Advantages and Disadvantages of Various Routes of Administration

Topical and oral route is an effective route for skin lightening. This is due to the presence of an amino acid called cysteine. This amino acid has been proven to lighten skin color in sun exposed skin because it helps in lowering the melanin production in your skin. By lowering your skin melanin production and by your skin producing lighter tones, your skin will begin to appear like its original tone without the sun blemishes. It has another great skin benefit, anti aging. The most common cause of skin aging is photoaging. Photoaging is caused by repeated exposure to UV rays from the sun. UV rays produce a substance called free radicals which damage our skin cells and causes early aging. For oral route, possible side effects may include allergic reactions, abdominal cramps and bloating in some patients and it should be avoided during pregnancy and breastfeeding.^[37]

Intravenous route is the most effective route for skin lightening and it provide fast results but it is not at all safe due to the reported side effects such as abdominal cramps, deranged liver functions, feeling heart sinking, diarrhea, anaphylactic shock and vomiting.^[31,32] The above mentioned advantages and disadvantages are general pros and cons for dosage 20-40mg/kg body weight per day and its effect varies from person to person. Due to a lack of research, little is known about the side effects and their function on different body. So, it is advisable that one should always consult their doctor before taking any kind of supplementation.

Legal Aspect

Glutathione based oral dietary supplements have been granted the status of "Generally recognized as safe",

consistent with Section 201(s) of the federal food, drug and cosmetic act of the United States Food and Drug Administration.^[38] There is no restriction on its availability in United States, Philippines and Japan. This has become available over-the-counter in India as well. As injectable glutathione is approved by FDA Philippines as an adjunct treatment in cisplatin chemotherapy but currently FDA warned compounders on February 1, 2019, not to use glutathione L-reduced powder distributed by Letco Medical located in Decatur, Alabama, to compound sterile injectable drugs due the adverse effects such as nausea, vomiting, lightheadness, chills, body aches and sneezing faced by seven patient who received an injectable drug compound with L-glutathione.^[39]

Safety Aspect

The safety aspect of Glutathione as a skin lightening effect in its oral and topical form has been considered safe due to the lack of any adverse effects by US-FDA but the safety aspect of intravenous glutathione is still questionable due to its adverse effects such as nausea, chills, body ache, and sneezing.^[38,39,40] Oral and topical glutathione is easily available and people can buy it easily from online and drug store without thinking too much about the side effects.

Future Development

S-acetyl-glutathione consists of oral glutathione attached to a sulfur atom. It is taken up intact by chylomicrons in the gut. The acetyl group prevents its oxidation and increases its plasma stability. Studies conducted in mice and human foreskin fibroblasts have revealed that S-acetyl-glutathione molecules are taken up directly by cells with subsequent conversion to glutathione by cleavage of the acetyl bond within the cell. This results in higher levels of intracellular glutathione.^[41] S-acetyl-glutathione is also known to have antiviral and immunomodulatory properties. However, there is no human data available to prove the superiority of S-acetyl-glutathione over plain glutathione for skin-lightening effects. Liposomal glutathione consists of the molecule encapsulated in water inside a fat ball with the intention of "tricking" the digestive system to interpret it as a fat cell. This prevents it from being hydrolyzed thereby allowing it to enter the bloodstream. However, the lacks of human trials, quick degradability of liposome and safety concerns of soy lecithin (a liposomal component) are barriers against its current use.^[42,43]

DISCUSSION AND CONCLUSION

Glutathione is said to be the natural antioxidant which has many benefits such as it reduce oxidative stress, reduce cell damage, may help against autoimmune disease, regenerating vitamin C and E and making DNA, etc. Topical glutathione as a skin lightning agent was an accidental discovery and it reduces tyrosinase activity and it is confirmed by a randomized, double blind placebo controlled clinical trial. The Three randomized controlled trials have been conducted to check the effect

of orally administered glutathione on the skin melanin index but with short term follow-up periods. These studies assist some skin lightening effects of oral, as well as topical glutathione. However more research data should be needed to support skin lightening effect of glutathione. There is no proper evidence to support intravenous glutathione as there is only a single clinical trial evaluating its efficacy and in that trial it was concluded that intravenous glutathione is not at all efficient due to its severe adverse effects have seen in patients. It is important to have more randomized, double-blind, placebo-controlled trials with a larger sample size, long term follow-up period, with well defined primary and secondary outcomes, targeted to evaluate the efficacy and safety of the skin lightening effect of topical, oral and parenteral glutathione. Many people take oral glutathione tablets even without a prescription, which can be dangerous because everybody is different and it react differently so it is recommended that one should consult doctor before taking anything internally. Topical glutathione is available in different forms such as creams, lotion, face washes, etc, but before using it patch test should be performed first because it could be allergic to skin and the bioavailability of glutathione is poor, it is not easily absorbed in body alone, it requires vitamin C for better absorption. It can be foreseen that in the future glutathione may play a significant role as an effective functional cosmetic ingredient but scientific studies and research is needed in the field of cosmetic applications.

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