Skin Lightening Treatments: A Review on the Effect of Intravenous Glutathione in the Disease States of Women

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Abstract

The skin bleaching industry is a global business with a vast array of anti-melanogenic choices including glutathione. Glutathione is synthesized *in vivo* but has been used as a bodily supplement by medical personnel to aid in preventative medicine. Known for its antioxidant properties, glutathione has been used for its anti-melanogenic effects. Intravenous glutathione requires more investigation to determine its safety for usage. It continues to be distributed to the cosmetic industry despite antagonism from the Philippine FDA. This study will research the potential effects of intravenous glutathione on women and it will propose the biochemical mechanisms of glutathione in induced disease states in women. The aim is to educate people about safer methods for skin lightening.

Keywords: skin lightening, intravenous glutathione, pheomelanin, Fitzpatrick skin types, Stevens-Johnson syndrome.

Skin Lightening Treatments: A Review on the Effect of Intravenous Glutathione in the Disease States of Women

Skin lightening, more commonly known as skin bleaching, is globally practiced by women who are of darker shades of skin. It is a form of body modification performed to alter appearance and temporarily alleviate psychological issues that stem from self-hatred and low self-esteem.[1] Racism and colonialism have attributed greatly to the psychological pressures of having lighter skin. Fairer skin is viewed as more attractive, tasteful and healthy.[2] Statistically speaking, women of African descent participate the most in skin lightening, with 70% of women in Nigeria, 30-40% of women in Pretoria, South Africa, 50% of women in Mali, and eight out of every ten women in the Ivory coast participating in this practice.[3,4] In a study done by Schroff et al., it was suggested that Indian women were generally two times more likely to use lightening products than Indian men. The statistics in Europe and Asia suggest 27-77% usage in different communities. The toxicity of the ingredients, such as mercury and hydroquinone, has been sufficient to ban the products from circulation in an open market in countries like Ghana, Zimbabwe, South Africa and Nigeria.[5] The constitutive skin types that lighten their complexion tend to fall under the Fitzpatrick skin types IV-VII; individuals with skin types that are I-III would normally lighten their skin in cases of hyperpigmentation, melasma, or acne scars that may alter the color of the areas affected.[6] The percentage of women who bleach their skin in the world correlates with the skin type distribution as illustrated by Table 1, which is based on modified figures from studies performed by Holcomb et al. and Bino et al.[10, 11] Manufacturers and skin lightening product distributors do not warn users of the dangers of lightening skin

because of how lucrative the industry is.[15] The product market is estimated to reach $8 billion by 2026 globally.[7]

Table 1

Fitzpatrick scale of skin types and their associated characteristics as they relate to the distribution of women who would practice skin lightening.

Fitzpatrick Scale			
Photo type	**Sunburn and tanning criteria**	**Constitutive skin color**	**Heritage of photo type prevalence (but not limited to)**
I	Always burn, never tan	Very light	Northern Europe, United Kingdom
II	Always burn, sometimes tan	Light	Europe, Scandinavia
III	Sometimes burn, always tan	Intermediate	Southern Europe, Central Europe
IV	Never burn, always tan	Tan	Mediterranean, Asia, Latino
V	Moderately pigmented	Brown	East India, Africa, Native Americans
VI	Heavily pigmented	Dark brown to black	Aboriginal, Africa

The agents for skin lightening are classified into different categories based on their mode of action on the skin. Hydroquinone and its derivatives arbutin and kojic acid, azelaic acid, mercury, and botanicals are classified as chemical tyrosine kinase inhibitors; niacinamide is a melanocyte transfer inhibitor; alpha and beta hydroxy acid are vitamin A derivatives and are classified as accelerators of epidermal turnover; vitamin C, vitamin E, ubiquinone, glutathione and coenzyme Q-10 are antioxidants; and topical corticosteroids are anti-inflammatory in nature.[8] These have been used as agents to halt the production of eumelanin, which causes the brown pigment in skin. Many African countries have banned the distribution and sales of skin bleaching creams containing these agents due to harmful side effects such as hypopigmentation, skin sensitization and melanoma.[9]

The effects of oral and topical applications of glutathione have been studied in higher proportions compared to intravenous glutathione. These studies have helped in establishing safety data. The Food and Drug Administration (FDA) of the Philippines declared dietary oral glutathione supplements as safe for use but have not approved the oral supplements in the case of skin lightening. Although the oral glutathione has not been declared safe to use, it is still manufactured and distributed. Due to the desire for an efficient treatment that is not localized to one part of the body the demand for intravenous glutathione has increased. However, due to limited information about the safety of intravenous applications of glutathione, organizations such as the Philippine Dermatological Society have issued a warning that the use of intravenous glutathione at high doses may be harmful to the recipient.[12]

Glutathione

Glutathione (GSH) is a ubiquitous, low molecular weight thiol, and tripeptide consisting of γ-L-glutamyl-L-cysteinyl-glycine. GSH has one of the greatest antioxidant properties and is important to normal cell function because it maintains cellular redox balance. Glutathione is abundant in cells but it is remarkably reduced in pathological conditions such as HIV/AIDS, Parkinson's disease, malnutrition, cancer, strokes, myocardial infarctions and others.[49] Glutathione is found in two forms, reduced (GSH) and oxidized (GSSG), as illustrated by Figure 1.[13] Glutathione is synthesized *de novo* from glutamate, cysteine and glycine through ATP hydrolysis. One oxidized glutathione molecule (GSSG) is made from two reduced glutathione molecules (GSH), which explains the higher ratio of GSH to GSSG intracellularly. Oxidative stress is indicated by a reduction in the ratio, as GSH has been used up to convert reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) to water, and by the decrease of NADPH, which reduces GSSG.

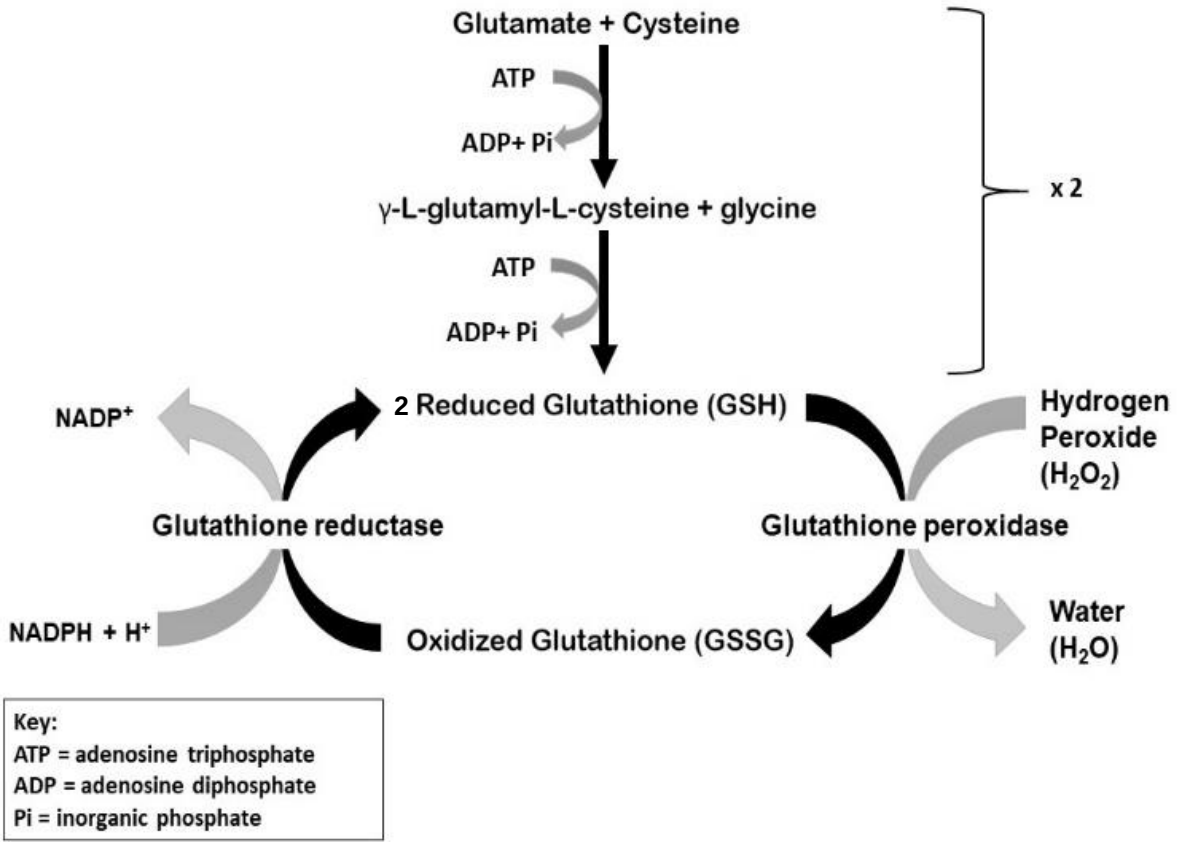

Figure 1. The glutathione redox cycle illustrating the function of the two glutathione variants. Figure 1 is adapted from a study performed by Sonthalia et al., and Ilkhani et al.[15, 16]

The ratio of these two forms of glutathione can indicate oxidative health. A normal ratio of GSH/GSSG is greater than 100, while oxidative stress is indicated by a ratio of 10 or less.[13] *De novo* glutathione synthesis is catalyzed by glutamate cysteine ligase and glutathione synthetase enzymes through a two-step process that requires ATP. GSH is found in large concentrations of 5 mM intracellularly.[13, 14] The most important functions of glutathione known to date are i) detoxification of xenobiotics, ii) catalysis of exchange reactions, iii) scavenging of free radicals and reactive oxygen species (ROS), iv) transport of amino acids across cell

membranes, v) acting as a coenzyme in some processes of cellular metabolism and vi) maintenance of thiol groups of proteins and other molecules.[15, 17]

Glutathione's popularity in skin lightening treatments comes as a result of its anti-melanogenic properties when it works as a tyrosinase inhibitor during melanogenesis (see Figure 2).[18] ROS directly activate tyrosinase. However, when GSH binds to the ROS, they are oxidized into a non-reactive form, inhibiting tyrosinase. GSH directly inhibits tyrosinase when its thiol groups react with the copper active site. Tyrosinase is mostly involved in the catalysis of dihydroxyindole (DHI) and dihydroxyindole carboxylic acid (DHICA) into DHI melanins and DHICA melanins, respectively. The presence of thiols such as GSH at the dopachrome stage of the pathway results in binding with dopaquinone to make thiolodopas. This pathway is favored due to the inhibition of the tyrosinases, causing the increased production of pheomelanins. Thiol groups found in GSH can directly inhibit tyrosinase by binding to its copper-containing active site. The production of the brown pigment eumelanin is halted, resulting in the switch to production of pheomelanin, which is a red/orange pigment.[20] This is one of three postulated mechanisms of action for GSH.

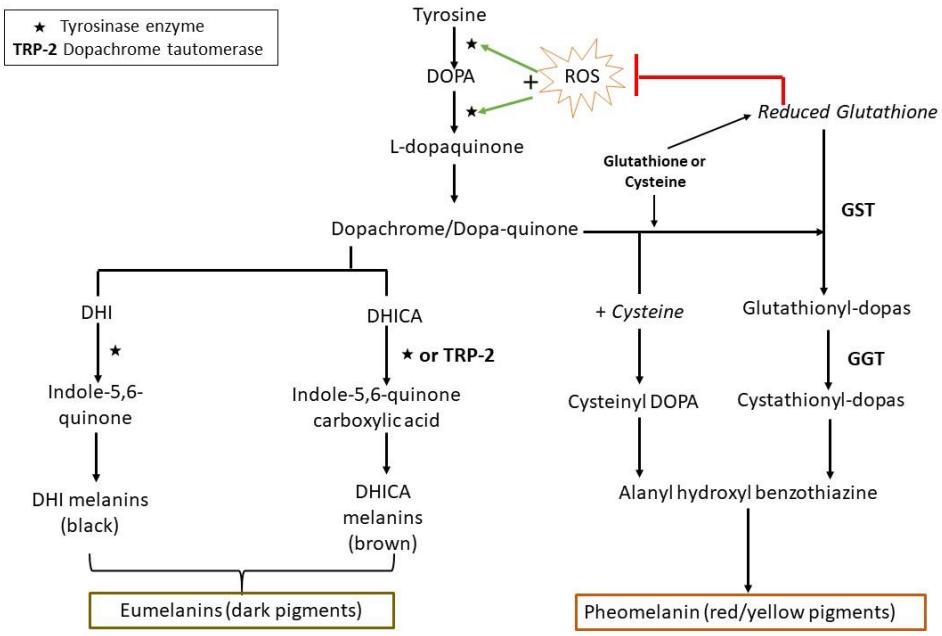

Figure 2. The Raper-Mason pathway shows the effect of the presence of GSH on the production of pheomelanin in melanogenesis. Figure 2 was adapted from studies by Sonthalia et al. and Davids et al.[15, 20]

Depleted levels of GSH in cells have been found in Parkinson's disease, cadmium exposure, HIV/AIDS, macular degeneration, and other neurodegenerative disorders.[13] Supplemental dietary GSH has been tested as a treatment for liver abnormalities, deficient immunity, pre-term infant autism, chronic otitis media, Parkinson's disease and other disorders. These supplements have been applied both orally and intravenously.[19] GSH is a thiol-containing compound. Its mechanism of action was inferred from past studies that linked thiol-containing compounds (kojic acid, arbutin, hydroquinone) to skin lightening. The skin lightening effect of these compounds was discovered during their use in a study for Parkinson's disease treatments.[20]

Compounds that act as scavengers for ROS, such as GSH, can slow down the effects of melanogenesis. Furthermore, if the compound has redox properties like glutathione, it can decrease melanin production by interacting with *o*-quinones or the thiol group at the active site of the enzyme tyrosinase.[21] The active site of tyrosinase contains Cu^{2+} and soft Lewis bases as thiol groups are inclined to chelate soft Lewis acids such as Cu^{2+}.[48, 52] Furthermore, when cysteine residues are present in active site pockets of tyrosinase, they cause disulfide interchanges with the thiol groups. A larger network of inhibition is created when cysteine residues increase around the tyrosine active site.[48]

Oral Glutathione

Topical creams are slowly fading away in usage as their effects are restricted to the area of application, making oral lightening treatments the alternative. Oral glutathione treatments have increased in popularity due to the impression that they give the whole body a lighter appearance. However, this is contrary to scientific research.[14] Torula yeast (*Candida utilis)* has been the main source of oral glutathione, which has been used as a dietary supplement. This derived glutathione has been the primary ingredient in supplements. Secondary ingredients include antioxidants like vitamin C.[15]

Dosing of oral glutathione has been inconsistent between manufacturers as a result of insufficient studies, making it difficult to assess the side effects of this treatment. Oral glutathione has been made available in the form of pills and solutions.[15] One manufacturer, Flawless Beauty and Skin, recommends a dosage of three pills that contain 900 mg of L-glutathione.[22] Minor ingredients include N-acetyl-L-cysteine (300 mg), alpha lipoic acid (225 mg), L-methionine (150 mg), vitamin E (150 IU), vitamin B2, (7.5 mg), and selenomethionine

(300 mcg). According to the manufacturer, these ingredients were combined to enhance glutathione serum levels in the body. This product emphasizes that it is not approved by the FDA even though it is manufactured in the United States of America.[22]

In comparison, an Indian manufacturer recommends a dosage of 10-20 mg per kg body weight for use as an antioxidant or anti-aging medication. In contrast, in the case of skin lightening, the recommended dosage is 20-40 mg per kg body weight. Furthermore, the duration of treatment is dependent on the client's skin color, with darker shades of skin requiring treatment for over two years, and lighter shades of skin needing one to three months of treatment until the desired outcome has been achieved. This product is recommended in combination with vitamin C (2000 mg per day) for the glutathione to be absorbed most efficiently. Each glutathione pill taken contains 1000 mg, and a client is prescribed one pill twice a day.[24] These two products are small examples of how diverse the industry is in dosage and duration recommendations. Indeed, there is no scientific basis for the recommended dosage.

Studies done by Kovacs-Nolan et al. on the fate of oral GSH have shown that it can cross the intestinal epithelium, but it is readily oxidized to GSSG.[25] GSH is quickly digested by gamma-glutamyl transferase (GGT) in the upper jejunum into its constituent amino acids: cysteine, glycine and glutamate.[26] It has been found that intracellular GSH is more effective when its constituent amino acids are taken on their own instead of GSH itself, as there is a higher concentration of GGT in the liver which further degrades the oral GSH that has been absorbed into the bloodstream after digestion. Oral cysteine is degraded in the digestive tract, but supplementing it in the form of N-acetylcysteine (NAC) increases the levels of cysteine because it is protected from degradation.[13] Oral glutathione has a low bioavailability, and this may be the

motive behind the pursuit of intravenous (IV) glutathione.[27] The estimated half-life of oral glutathione in plasma is 1.6 min.[39] Additionally, it should be noted that any skin lightening treatments sought from this method are temporary as pheomelanin production is low and inconsistent.[28]

Intravenous (IV) Glutathione

IV glutathione has been recently introduced in the cosmetic industry to speed up the skin lightening process, and to have long-lasting effects. This method is controversial due to the lack of safety data regarding its use. Few research studies have been performed to date concerning IV glutathione despite many women, especially in the Philippines, using this as their primary skin lightening agent.[15] The Philippine FDA released a statement warning of the toxic consequences to the nervous system, liver and kidneys and the development of the rare Stevens-Johnson syndrome with the continued used of IV glutathione. The greatest concern from the FDA is the incorrect administration of this treatment by untrained and non-medical personnel. In the black-market areas that provide this service safety precautions may not be observed. The use of injections and a drip in non-sterile conditions can lead to the transmission of HIV as needles are shared, and the spread of different types of hepatitis. Overall, clients can experience embolisms caused by the introduction of intravenous air bubbles, and sepsis caused by pathogens and counterfeit glutathione.[29] On a cellular level, increased serum levels of intravenous glutathione can lead to reductive stress that is just as toxic to the body as oxidative stress.[32]

Research Question

Despite insufficient data to support the use of glutathione as a skin lightening treatment, it is still used. This study will investigate the ability of glutathione to lighten skin. First, the

mechanism of action that glutathione takes to lighten skin will be investigated to bridge the gap that exists between topical, oral and IV administration. Then, the effects of oral and topical glutathione in the body will be investigated by studying their biochemical mechanisms to compare them to IV glutathione. This study will also explore the side effects of glutathione when used for its melanogenic properties. Finally, by looking into the pathogenesis of disease, possible effects of IV administration of glutathione will be mapped to determine the potential long-term consequences of skin lightening.

Literature Summary of Glutathione Skin Lightening Products

IV glutathione has not been officially recognized as a skin lightening agent. Its mode of action has not been observed. Furthermore, only a few studies have been conducted to date to observe the efficacy of oral and topical glutathione in skin lightening. The following studies were published over four years ago, and there is a need for more recent data as glutathione is increasing in popularity. Table 2a and 2b are a compilation of known studies that may aid in a proposal of a mechanism of action. These tables are adapted from Table 1 published from the study by Sonthalia et al.[15]

Table 2a

Investigations of glutathione as a skin lightening treatment topically and orally.

Type of Glutathione Application	**Topical**	**Oral (capsules)**	
Study Reference	Watanabe et al. [22]	Weschawalit & Asawanoda [44]	Arjinpathana and Asawononda [46]
Year	2014	2016	2010
Subject Information	30 healthy Filipino females; tan; Fitzpatrick skin types III or IV; 30-50 years of age	60 healthy females randomized into 3 groups; 20-50 years of age	60 healthy medical school students; 18 males and 42 females; 19-22 years of age
Study Design	Randomized, double-blind, placebo-controlled, matched-pair study	Randomized, placebo controlled, three-arm study	Randomized, double-blind, placebo-controlled, two-arm study
Methods Applied	0.5 g of lotion comprised of 2% (w/w) GSSG or placebo without GSSG randomly assigned and spread evenly to either	250 mg/day of GSH, 250 mg/day of GSSG or placebo (dibasic calcium phosphate), randomly assigned to subjects	250 mg glutathione or placebo capsules taken twice daily on an empty stomach for 4 weeks, block-

	left or right cheek of each subject		randomized assignment to subjects
Parameters Evaluated	Measured every 2 weeks for 10 weeks: 1) Skin whitening using Mexameter for melanin index (mexameter is a tool used to measure melanin and hemoglobin levels through absorption/reflection of light) 2) Skin moisture 3) Skin firmness using a Triplesense TR-3 sensor device 4) Efficacy against wrinkle reduction by observing “crow’s feet” 5) Skin smoothening	Measured every 4 weeks: 1) Melanin index 2) Presence of UV spots 3) Transepidermal water loss 4) Water contents 5) Elasticity	Measured at baseline and after 4 weeks: 1) Melanin index using a Mexameter: *Sun exposed areas*: i) Face (both sides), 2.5 cm caudally from the lateral canthi ii) Extensor surfaces of both forearms, 7 cm above the ulnar styloid processes *Sun protected areas*: i) Upper, inner arms, 10 cm from axillary vault ii) Standardized photographs were taken using a VISIA CR system to

			quantitatively measure: a) UV spots b) Pores c) Skin evenness
Subject Self-Evaluation	Scoring pattern used to determine skin whitening and smoothening: -3 = marked deterioration -2 = moderate, visibly uneven deterioration -1 = slight deterioration 0 = no apparent change/ improvement 1 = slight change/improvement 2 = visible change/improvement (<50% lightening of skin color)	No subject participation in evaluation	Used global assessment by scoring with a 4-point scale: 4 = very satisfactory 3 = moderately satisfactory 2 = minimally satisfactory 1 = not satisfactory

	3 = marked improvement/ significant change (uniform skin whitening, >80% of application area)		
Safety and Side Effects Listed	Well-tolerated; all subjects completed study; 1 subject had mild erythema on days 2 and 3; no reports of unwanted symptoms	Well-tolerated	Well-tolerated; 1 subject in glutathione group experienced flatulence; 1 subject in placebo group experienced constipation
Follow-up	None after 10 weeks of study	None after study duration	None after study duration
Study Results	1) Skin lightening melanin index: A) Placebo - decreased slightly after 10 weeks (Week 0: 274.13 [+/-] 25.81 vs. Week 10: 265.50 [+/-] 25.82)	1) Both GSH and GSSG caused decreased melanin indices 2) GSH more effective in wrinkle improvement 3) GSH and GSSG increased skin elasticity in	1) GSH group had greater decrease in melanin index than placebo group on sun-exposed areas 2) Smaller number of UV spots developed in subjects on

	B) GSSG lotion – decreased after 10 weeks (Week 0: 272.77 [+ or -] 26.17 vs. Week 10: 243.47 [+ or -] 26.31) 2) Moisture index values were higher at GSSG sites than placebo sites 3) Curvature and keratin values were significantly lower in GSSG sites than placebo sites 4) Elasticity values did not show a remarkable difference between placebo and GSSG lotion	both sun exposed and sun-protected skin	glutathione treatment; an increase in skin evenness and a reduction in pore size were reported with the treatment 3) Subjects reported an average score of 3.06 for satisfaction
Limitations	Small study size, short duration and no follow-up; limited to women of Fitzpatrick skin type III-VI; results may be	Study had insufficient data to analyze limitations	Plasma glutathione levels not measured; study duration was short and no follow up

	limited to Filipino women		
Proposed Mechanism of Action	A) Conversion of GSSG to GSH in epidermis B) GSH activates pheomelanin pathway	Unavailable	GSH activates pheomelanin pathway

Table 2b

Investigations of glutathione as a skin lightening treatment orally and intravenously.

Type of Glutathione Application	**Oral (buccal lozenges)**	**Intravenous**
Study Reference	Handog et al. [45]	Zubair et al. [32]
Year	2015	2016
Subject Information	30 Filipino females who work as medical personnel, Fitzpatrick skin types IV – V, with melanin indices of ≥ 20 out of 99; 22-42 years of age	50 female test subjects enrolled, 32 had data recorded from them and were divided into 2 groups: Group A skin types match group B skin types; 25-47 years of age
Study Design	Open label, singe–arm pilot study	Placebo-controlled
Methods Applied	Identical bottles containing 30 lozenges (500 mg of GSH) were given to each subject	Group A: 16 subjects given IV glutathione and vitamin C (injection of GSH Detox Forte ®, 1200 mg)

	Subjects kept one lozenge in their mouth, against their inner cheek (buccal mucosa) every morning, until completely dissolved	Control Group B: 16 subjects given IV saline as placebo (injection of 5 mL saline, 10 mL distilled water) 2 unexposed body sites were chosen (upper inner arm below axilla and upper outer thigh of all patients).
Parameters Evaluated	Evaluation done every 2 weeks for 8 weeks: 1) Portable Mexameter used to measure melanin index 2) Melanin indices taken from sun-exposed area (extensor surface of wrist) and sun-protected area (mid sternum) 3) Liver function tests, serum glutamic pyruvic transaminase (SGPT), complete blood count (CBC) and serum glutamic oxaloacetic transaminase (SGOT) were done at baseline	Administration for 6 weeks and 2 independent observers evaluated: Skin tone measured with Taylor hyperpigmentation scale (tool consisting of 15 uniquely colored plastic cards with hues that apply to Fitzpatrick skin types I-IV and is used to visually assess complexion), cards are used to analyze change from darker shades to light shades [47]

Subject Self-Evaluation	Used global assessment scores to determine skin lightening: None = 0 Mild change = 1 Moderate = 2 Obvious = 3 Very marked = 4	No subject participation in evaluation
Safety and Side Effects Listed	Well-tolerated; 29 out of 30 subjects completed the study; 30th subject complained of soreness in gums due to lozenge administration; another subject complained of chalky taste but completed study. CBC, SGPT and SGOT values remained normal at week 8.	Not well-tolerated; 32 out of 50 completed study; 8 subjects from group A had deranged liver function tests (defined as 1.5 times above reference range), and 1 subject developed anaphylactic shock; all were excluded from results. 9 control from group B who matched the skin types of subjects excluded from group A were also excluded to keep study standardizations Side effects in 25 subjects in group A: Feeling warmth during injection (44%) Abdominal cramps (40%)

		Abnormal liver functions (32%) Feeling of heart sinking (28%) Diarrhea (16%) Paresthesia (16%) Dizziness (12%) Anaphylactic shock (4%) Vomiting (4%)
Follow-Up	None after the duration of study	2, 4, 6 months after end of administration
Study Results	1) In sun-exposed area: At week 8, 100% of subjects experienced a reduction in their melanin indices ($p<0.0001$) from week 0; significant skin lightening effects only showed after week 2 2) In sun-protected area: At week 8, 100% of subjects experienced a reduction in their melanin indices from week 0 3) 27/30 subjects (90%) evaluated their skin as having undergone	1) GSH group A skin improvement, n=16 0 months (after 12 injections): 6 (37.5%) 2 months: 3 (18.7%) 4 months: 3 (18.7%) 6 months: 1 (6.2%) 2) Placebo group B skin improvement, n=16 0 months (after 12 injections): 3 (18.7%) 2 months: 2 (12.5%) 4 months: 0 (0%) 6 months: 0 (0%) Not very effective for skin lightening

	moderate lightening (score 2 out of 4); 3/30 subjects (10%) evaluated their skin having undergone mild skin lightening (score 1 out 4)	
Limitations	Small study population and duration No control used and no follow-up Blood GSH levels were not measured	Blood GSH levels were not measured Taylor hyperpigmentation tool is more useful when used subjectively but a Mexameter is more useful to measure melanin index objectively and accurately [50] Small study population and short duration
Mechanism of Action	GSH used mucosal route to get to epidermis all around the body	GSH absorption increased when administered with vitamin C

Analysis of the Literature

Most of the above studies were conducted in Southeastern Asia countries, where residents usually have the Fitzpatrick skin types III-IV and olive/tan skin (Table 1). The results displayed that glutathione in general does help with skin lightening, but the most effective treatment was through oral glutathione administration. Oral administration also appeared to be the safest because it had minor or no side effects on the subjects compared to IV glutathione.

GSH and GSSG both had the same effect in reducing the melanin index of the skin. This would be expected because GSSG can be easily reduced into GSH by glutathione reductase (see Figure 1) to be used as GSH in the Raper-Mason pathway. These studies suggested that glutathione has skin lightening properties when applied topically and orally, and the proposed mechanism was through the shifting of the Raper-Mason pathway to produce pheomelanin instead of eumelanin. According to the study done by Zubair et al., IV glutathione does not appear to retain its skin lightening properties over time.[32] The missing link in the studies was the lack of data pertaining to serum glutathione levels. By monitoring the levels of GSH in the blood, the metabolism of IV glutathione may be investigated, and mechanisms of action proposed.

Proposed Disease States Directly and Indirectly Correlated to IV Glutathione

The study done by Zubair et al. in 2016 to test the efficacy of IV glutathione against a placebo in skin-lightening is one of the only studies published to date. The data from this experiment is helpful because it gives the potential effects of using IV glutathione over long periods of time. Of all the side effects, notably 40% of volunteers from group A experienced abdominal cramps, 32% reported deranged liver functions and 16% reported diarrhea and paresthesia. The control group B did not record any adverse side effects, which suggests that intravenous glutathione was the cause for the side effects. The main objective of the experiment was to observe changes in skin color: 37.5% of women were observed to have a change in skin color immediately after the completion of 12 injections. By the last observation at six months, only 1 out of the 16 women who completed the treatment showed a consistency in skin color improvement.[32] Nine women had to stop treatment due to adverse side effects. Eight out of the

nine women experienced issues with their livers, and one suffered from anaphylaxis due to the treatment (Table 2b).

Reductive Stress Can Cause Oxidative Stress

Reductive stress is the opposite of oxidative stress and happens as a result of the redox imbalance of the species GSH/GSSG, nicotinamide adenine dinucleotide (NAD^+)/NADH and phosphorylated NAD^+ ($NADP^+$)/NADPH; these species are important in the maintenance of a homeostatic environment for cells. An increase in the formation of GSSG is a marker of oxidative stress.[33] Increased levels of intracellular GSH can induce pro-oxidant activity and halt its antioxidant properties towards ROS.[34, 35] Reductive stress is known to mostly contribute to inflammatory disease in contexts such as cancer, pulmonary hypertension and hypertrophic cardiomyopathy.[33] ROS are beneficial in the body for redox homeostasis, and well-functioning cardiovascular and immune systems. For example, in redox regulation, the ROS react with the amino acid cysteine on proteins, which is important in signaling pathways. ROS are also important in the cell cycle and in influencing apoptosis. ROS can bind to the mitogen-activated kinase, which triggers the apoptotic cascade.[51] Consistent reductive stress caused by a shortage of ROS can cause oxidative stress as a result of negative feedback: redox proteins can donate excess electrons to O_2 which generates ROS such as superoxide (O_2^-). An increased concentration of ROS leads to oxidative stress, which triggers increased action by the ROS scavengers, and may trigger a reductive stress crisis again.[36] This cycle can cause cellular damage as homeostasis is disrupted.

Increased production of pheomelanin. GSH is imperative for the melanogenesis pathway to produce pheomelanin, and its continued production is sustained by high levels of

cysteine that interact with the DOPA-quinone as illustrated in Figure 2.[43] The use of IV glutathione, as demonstrated by the study conducted by Zubair et al., can alter skin color. However, the changes were temporary.[32] People with lighter skin types (Fitzpatrick skin types I-III) are 70 times more at risk to develop skin cancer compared to those who fall under the Fitzpatrick skin types IV-VI.[37] Eumelanin is regarded as the more protective of the two types of melanin as it is not implicated in melanogenesis as much as pheomelanin.[37] While the exact mechanism is unknown, pheomelanin production increases ROS in melanocytes, and therefore decreases glutathione stores within the cell. The introduction of high levels of intravenous GSH can help to recover depleted levels of the antioxidant in the cell and maintain the level of cysteine for pheomelanin production, rather than exacerbate oxidative stress in melanocytes.[38]

A study performed by Nasti and Timares about the roles of eumelanin and pheomelanin in the susceptibility to skin cancer concluded that there was a correlation between different strains of mice (agouti and yellow coats) with high pheomelanin production and a higher risk of developing melanoma.[37] More research needs to be done to determine the direct link between pheomelanin and melanoma, and how other factors such as UV radiation contribute to it.

It has been found that GSH is vital for metastatic melanoma growth. In a study done by Carretero et al. to investigate the link between glutathione content and the activity of metastatic liver cancer cells using B16 melanoma cells, it was observed that GSH regulated energy metabolism and increased cell growth.[42] GSH is produced *in vivo*, but low levels of cysteine cause a lag in GSH synthesis. When cysteine was supplemented in this study, growth of the B16 melanoma cells was expedited, as cysteine is the rate-limiting step in GSH production.[42]

Exogenous intravenous supplementation of glutathione could possibly increase cancer growth as it may supply the much-needed cysteine when GSH is broken down intracellularly.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). These are rare, life-threatening conditions that are caused by an adverse immune reaction to medications. Medications for these conditions include anticonvulsants, allopurinol, sulfonamides, antibiotics and non-steroidal anti-inflammatory drugs. SJS and TEN physically manifest themselves in the form of mucocutaneous blisters that may result in epidermal detachment and tissue necrosis.[30] Skin lesions appear, followed by symptoms that resemble a respiratory infection such as fever, headache and sore throat. People who suffer from HIV/AIDS, lupus, or compromised immune systems are usually more susceptible to suffering from SJS and TEN.[31] The Philippine FDA mentioned that the use of intravenous glutathione can cause SJS/TENS. The mechanism of how this could occur is still under investigation, and little is known about the role of glutathione in this disease state.

Theoretically, glutathione may increase the risk of SJS/TEN occurring through cellular redox imbalance. High levels of ROS that could be caused by the reductive stress/oxidative stress cycle trigger apoptosis to occur. There are different mechanisms of the pathogenesis of these diseases, but one that may involve glutathione is keratinocyte apoptosis, followed by necrosis.[40] Apoptosis of the cell occurs as a result of damage to the mitochondria. The ROS may activate nuclear transcription factors such as tumor necrosis factor alpha (TNF-α) to begin the early stages of apoptosis, or indirectly cause apoptosis through cell damage by disrupting plasma membrane lipids and mobilizing Ca^{2+}, which leads to early apoptosis. Increased levels of GSH disrupt the ionic gradient of the mitochondria, causing it to rupture, leading to late necrosis.[41]

This is only a suggested mechanism to explain how increased glutathione serum levels may be related to SJS/TEN. More experimental data needs to be compiled as there is no definitive link to SJS/TEN, except the report from the Philippine FDA. The novelty of IV glutathione being used as a skin lightening agent is an opportunity for more experimentation to take place to investigate its effects.

Conclusion

This literature review has established that women, more than men, undergo skin lightening treatments. This is evidenced by most of the studies being conducted on women, and women having a stronger desire to achieve a sense of beauty by having a lighter complexion.[5] The pursuit of lighter skin is not only fueled by current beauty standards, but also social acceptance and higher social status. The Fitzpatrick scale in Table 1 showed that the photo types IV-VI are more likely to undergo skin lightening treatments, which correlates with the certain regions in the world such as Asia, East India, Southeastern Asia and Africa.

Although supplemental oral glutathione has been declared as safe, oral glutathione for skin lightening reasons has not been approved by the Philippine FDA. Use of the intravenous version of this skin lightening treatment has been warned against by the Philippine FDA due to the adverse side effects reported. Despite this, intravenous glutathione is still sold on the market, and still being used. Using glutathione could be risky if it is administered by untrained personnel. Dangerous side effects may result, including the transmission of HIV and different forms of hepatitis from recycled needles. Furthermore, there is an inconsistency with the dosing of glutathione for skin lightening among manufacturers, which may pose a danger to users as excessive glutathione may lead to liver toxicity. Studies exploring the anti-melanogenic effects

of glutathione are few, and those reporting data are limited to Southeastern Asian women. Women who use skin lightening treatments may use glutathione (topical, oral or IV) over long periods of time, and unfortunately studies to date were too short to determine long-term consequences.

The mechanism of action of glutathione established from research is the inhibition of the tyrosinase enzyme. The inhibition of the tyrosinase active site shifts eumelanin production to pheomelanin. The injection of IV glutathione has been shown to raise serum GSH, which could lead to reductive stress as there is a large concentration of GSH in cells. By negative feedback, this could result in oxidative stress. Theoretically, increased ROS may be the reason behind the aggravation of conditions such as SJS/TEN. Increased pheomelanin production may cause the user to be at higher risk of suffering from melanoma. This study was inconclusive in establishing a direct link between IV glutathione and SJS/TEN.

GSH is manufactured *de novo* because it is important for bodily functions. Normal cellular actions require GSH to prevent tissues from undergoing oxidative stress as it is the greatest antioxidant. Since it is already found in high concentrations in the body, its supplementation at high doses could be toxic for the body, especially if taken in unregulated doses. The use of glutathione is not based on scientific findings and could eventually cause more harm than good. Oral glutathione may have been established as a lightening product, but intravenous glutathione is not effective in skin lightening and may lead to redox crises in the body with continued use.

Future Experimentation

The literature in Table 2 showed that none of the experiments monitored GSH serum levels which could play an important role in outlining the mechanism of glutathione metabolism. Since glutathione is made up of cysteine, glutamate and glycine, one of these amino acids could be tagged. The half-life of glutathione is short because it is digested to its constituent amino acids. Cysteine could be tagged as it is imperative in the production of pheomelanin. The urine of subjects could then be monitored for tagged cysteine molecules to observe if GSH was excreted due to excess levels in the blood. More experiments need to be done on topical, oral and intravenous methods to establish safe doses and to monitor the effects of taking glutathione for long durations. Finally, the Philippine FDA suggested the occurrence of SJS/TEN with the use of IV glutathione. Although rare, it is theoretically feasible, and a study could be conducted to determine if glutathione directly causes this condition, and if reductive stress plays a role.

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