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Exploring the Dual Benefits of 'TOMESORAL': Photo-Protective and Skin Lightening Potential of Carotenoids and Flavonoids in a Preliminary Controlled Trial

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Abstract
The concept of photoprotection by dietary means is gaining attraction. Plant constituents such as carotenoids and flavonoids are involved in protection against excess light in plants and also contribute to the prevention of UV damage in human skin. The water soluble flavonoids are the compounds that potentially elevate the levels of glutathione. In this study, TOMESORAL+ white tomato powder (rich in phytoene, phytofluene, lutein and zeazanthine) was evaluated for its potential to protect the skin from UV and increased glutathione levels. Study comprised of evaluation of skin lightening effect in post treated groups of skin type III or IV. Evaluation of reduction of skin stress and improvement of skin regeneration by the estimation of ELISA based markers. Similarly, study of reduction of inflammatory markers in post treated skin groups was also done. TOMESORAL treatment group expresses low levels lipid peroxidation and inflammation making TOMESORAL a potential option for treatment of inflammation due to photo-damage and improve the skin health. Moreover, the unique natural cocktail of carotenoids and flavonoids in TOMESORAL augments the increase of glutathione and glutathione reductase levels in photo-damaged skin that not only repair the damaged skin but also contribute to enhance the skin resistance against the damaging effects of UV radiations.

Keywords: TOMESORAL, inflammation, ELISA, photoprotection, carotenoids and flavonoids

1. INTRODUCTION

The content and distribution of different pigment substances, such as melanin, haemoglobin and carotenoids, is the main determination of human skin colour (Costin and Hearing 2007). The main colour of the skin is determined by genetic elements, but also by acquired factors. Melanin is produced via an amino acid known as L-tyrosine, in melanosoma, which is melanocyte organelles, metabolised by a series of enzyme reactions. There are several types of melanin, like eumelanin and pheomelanin, which give different colours (Schiaffino 2010). Melanosomes supply the surrounding keratinocytes with melanin, which results in melanin spread throughout the skin and expressed to produce different skin colours (Cardinali, Ceccarelli et al. 2005). Melanin also plays an important role in protecting the body against toxic UV rays, in addition to their effects on the skin's visual appearance (Epstein 1983). The metabolism of melanin in the skin has thus been an important topic of research both physiologically and aesthetically (Slominski, Kim et al. 2014, Slominski, Zmijewski et al. 2015).

An abnormal melanin metabolism may lead to different kind of pigmentation and hypopigmentation disorders (Slominski, Tobin et al. 2004, Giehl and Braun-Falco 2010). Hyperpigmentation occurs when, due to inflammation, ageing, UV, physical damage and other internal/external stimular factors, melanin accumulates excessively or unevenly (Rose 2009, Callender, Surin-Lord et al. 2011). In the meantime, genetic or epigenetic defects may lead to hypopigmentation, as albinism or vitiligo in melanin production (Ganju, Nagpal et al. 2016, Spritz and Andersen 2017).

Photo-protection, pharmacotherapy, surgical (chemical peeling and laser treatment), and cosmetic camouflage are part of prevention and treatment strategies for hyperpigmentation (Levy and Emer 2012, Saxena, Andersen et al. 2015, Pavlic, Brkic et al. 2018). Chemical peeling and laser treatment are common but have side effects, like dermatitis or recurrent pigmentation (Chu, Chou et al. 2015, Borelli, Ursin et al. 2020). Hydroquinone is mainly used as a pharmacotherapeutic but may cause irritation of the skin, allergies, mutations and cancer (Jow and Hantash 2014). In the field of cosmetics, skin lightening is dominated by functional cosmetics containing arbutin, niacinamide and vitamin C derivatives, while the safety and efficacy of consumers is low (Desmedt, Courselle et al. 2016). In the field of cosmetics, skin lightening is dominated by functional cosmetics containing arbutin, niacinamide and vitamin C derivatives, while the safety and efficacy of consumers is low (Kim, Park et al. 2012, Park and Boo 2013, Kwak, Seok et al. 2016, Lee, Kim et al. 2019).

Our study shows that TOMESORAL white tomato powder rich in flavonoids (like quercetin and rutin) and carotenoids (phytoene (PT), phytofluene (PTF), lutein and zeaxanthine) could be a potent composition that have many health and cosmetic

benefits including the potential to protect against UV (both UVA and UVB) and enhances the production of gluthathione synthesis enzymes that will in turn enhances the production of reduced glutathione. PT, PTF, lutein and zeazanthine proved to protect the skin from UVA and UVB (Sujak, Gabrielska et al. 1999, González, Astner et al. 2003, Aust, Stahl et al. 2005, Meléndez-Martínez, Stinco et al. 2019). The water soluble flavonoids are the compounds that potentially elevate the levels of glutathione (Myhrstad, Carlsen et al. 2002, Yang, Lii et al. 2011). In case of TOMESORAL white tomato powder, phytoene, phytofluene, lutein and zeazanthine could potentially protect the skin from UVA and UVB. On the other hand, PT and PTF are not responsible for increasing the glutathione level; instead the water soluble flavonoids and other compounds are major player in increasing the glutathione levels after absorption from small intestine.
The primary objective of this study was to evaluate the photo-protective effects of TOMESORAL food supplement in volunteers with photo damaged skin. Moreover the product was also evaluated for reduction of fine lines, wrinkles, skin stress, lipid peroxidation and improvement in the production of reduced glutathione alongwith skin tone.

METHODOLOGY

Trial period
Products reception: September 29, 2020
Beginning of the study: October 15, 2020
End of the study: December 10, 2020
This was a double blind, placebo controlled trial and approved by institutional review board (IRB).

Assessment criteria
Primary criterion
Clinical assessments of the skin of the face and dorsal hands were performed for all the participants at 0, 4 and 8 weeks of the product use. The following parameters were assessed at each visit: fine lines and wrinkles, overall clinical grade of skin lightening, and tactile roughness. For the measurements of fine lines, wrinkles and overall clinical grade of skin lightening, the close-ups were taken from digital single-lens reflex camera (DSLR) (Nikon, D7500), and images were quantified with the help of Image J. Tactile roughness and skin regeneration were estimated on a clinical photo-numeric scale of 0-8.

Secondary criteria
- Study of skin lightening effect in post treated groups of skin type III or IV.
- Evaluation of reduction of skin stress by the estimation of ELISA based stress markers.
- Study of improvement of skin regeneration by different markers based upon ELISA.
- Study of reduction of inflammatory markers in post treated skin groups.
- Analysis of the subjects' answers to a subjective evaluation questionnaire.

Principles and measurement instruments
Assessment of skin health
Clinical assessments of the skin of the face and dorsal hands were performed for all participants at 0, 4, and 8 weeks of product use. The following parameters were assessed at each visit: fine lines and wrinkles, overall clinical grade of skin lightening, and tactile roughness. The degree of fine lines, wrinkles, and the overall level of skin brightening were scored according to the Griffiths photo-numeric scale for photo-aged skin assessed by an authorized dermatologist panel (Watson, Ogden et al. 2009). Further, measurements of fine lines, wrinkles and overall clinical grade of skin lightening were done by image quantification with the software Image J.

Subjective evaluation questionnaire
A subjective evaluation questionnaire prepared by the clinical trial experts was completed by the subjects at the end of the study to subjectively evaluate the organoleptic characteristics of the studied products and their future use.

Subject selection
Number of subjects
102 out of 132 healthy volunteers (men (47) and women (55)) were recruited for this study who met the study inclusion criteria (age range 30–60 years). The major points in inclusion for this study include:
1. All the participant informed that they are exposed to sunlight (either direct sunlight or day light in open air areas) for 5-6 hours a day.
2. These participants were evaluated by expert for the degree of photo-damage and designated as skin type I /II /III /IV.
3. Majority of the test participants were evaluated for recruitment as skin type III or IV.
4. All the test participants have showed the sign of fine lines or wrinkle, or both.
5. All the test participants have a healthy dietary schedule as wet by the nutritionist.
6. All the test participants gave us consent that they have not used any drug or antibiotic 2-3 months before the time of treatment**.**

20 healthy volunteers were also recruited as normal control subject. All the control participants also gave us the consent that they have not used any drug or antibiotic 2-3 months before the time of the study. This group was not given any type of

treatment and their skin type was marked by dermatologist as type I or II (fairer skin tone with less photo-damage).

Inclusion criteria

General criteria
Healthy subject.
Subject given his/her informed, written consent.
Cooperative subject, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the clinical trial center could have been expected.
Specific criteria
Sex: men and women
Age: between 30 and 60.
Person agreeing not to change its alimentation habits.
Person with type III or IV skin tone on the face (normal persons with type I or II skin tone)
For women with substitutive hormonal treatment: stabilized treatment since 12 month and more.
For women in age for procreation: use of an efficient method of contraception since 12 weeks before the beginning of the study, during the study and 4 weeks after the end of the study.

Exclusion criteria

Pregnant or nursing woman or woman planning to get pregnant during the study.
Cutaneous pathology on the studied zone (eczema, etc).
Use of topical or systemic treatment during the previous weeks liable to interfere with the assessment of the acceptability of the studied product.
Taking of a food supplement two month before the study.
All medical treatments taken continually 7 days before the beginning of the study.
All medical treatments taken during 2 weeks and more in continue the month before the beginning of the study.
Person enrolled in another clinical trial during the study period.
Known allergy to a constituent.
Severe chronic or acute problems to health

Compliance assessment and restrictions during the study

If the volunteer deviates the protocol or if the deviation was major, the volunteer was declared non-compliant. In this case, the subject was removed from the study for non-compliance. If the protocol was not respected and if the deviation was minor, the technician or the investigator warned the volunteer. No use of skin medications (pharmaceutical or cosmetic) products other than the studied products was authorized on the face during the study. Only the usual cleansing products were authorized on the face during the study.

Assessment of Vital functions

Assessments of the shock index (SI) (heart rate/systolic blood pressure; normal range, 0.5 to 0.7) and conventional vital functions in all participants at Week 0, week 4 and week 8 were done. These include Vitamin D levels, fasting glucose levels, total and direct bilirubin levels, alkaline phosphatase (ALP) levels, hepatitis B and C, heart rate and blood pressure.

Treatment Groups

Volunteers were divided into five groups excluding the normal ones. These groups' details are given in the table below:

No	**Abbreviation**	**Group name**	**Number at week 0**	**Number at week 8**
1	Normal	Persons with skin photo-type I and II	20	15
2	Untreated	Skin Photo-type III and IV with no treatment	20	16
3	T-Plac-250mg/day	Treatment with rice powder 250mg/day	20	15
4	T-Tomes O-250mg/day	Treatment with Tomes-Oral 250mg/day	20	15
5	T-Plac-500 mg/day	Treatment with rice powder 500mg/day	20	16
6	T-Tomes O-500mg/day	Treatment with Tomes-Oral 500mg/day	20	17

At the end of the treatment data in each group was restricted to 15 volunteers in each group. This reduction was due to the fact that 15 is the lowest number of respondents in some groups.

Trial Schedule

All test products were delivered in containers which were identically packaged and coded so that researchers and subjects were not aware of the treatment. Subjects were randomly allocated to self-treatment with either the vehicle formulation (rice powder capsules) or the test product as described by a randomization program (StatsDirect Ltd, Altrincham, U.K.) and instructed on the use of their allotted supplemented capsules — 250mg/day or 500mg/day for 8 weeks. Clinical assessments via biochemical testing were done at day zero, day 28th (4 weeks) and day 56th (8weeks) for all the participants to assess their vital function. Universal College Lahore Local Research Ethics Committee approved the study and all the subjects gave written, informed

consent. Blood sampling and morphological examination were done at day zero, 4 weeks and 8 weeks post treatment with Tomes-Oral capsules. Blood serum was subjected to ELISA for anti-inflammatory and regenerative markers. Skin tone was checked between different treatment groups in post treatment examination. Overall anti-oxidative enzymes status of the body was also assessed from serum samples.

Assessment of skin health

Clinical assessments of the skin of the face and dorsal hands were performed for all participants at 0, 4 and 8 weeks of product use. The following parameters were assessed at each visit: fine lines and wrinkles, overall clinical grade of skin lightening, and tactile roughness. The degree of fine lines, wrinkles, and the overall level of skin brightening were scored according to the Griffiths photo-numeric scale for skin type III or IV assessed by an authorized dermatologist panel (Watson, Ogden et al. 2009). The scale ranges from 0 to 8, where 0 represents very fair skin tone and 8 represents the dark skin tone. Skin types for these scale included Type I to Type IV, where type I represents fair skins and Type IV represent dark skin. Likewise, tactile roughness was scored on the random areas from 0 to 8, where 0 represents totally smooth skin with no rough patches and 8 represents much roughened skin.

Stress calculation

Stress is basically a body's response against any damage. During photo-damage (by UVA or UVB or both), the skin is considered to be stressed one that will ultimately resulted in lipid peroxidation of the skin's outer layer. This phenomenon further causes a marked reduction in anti-oxidants. Evaluation of lipid peroxidation could be done by MDA assay. Moreover, estimation of reduced glutathione (GSH) can be evaluated for the assessment of the lightening effect one the skin post-treated groups.

Estimation of MDA

For the evaluation of MDA in pre-treated and post-treated groups of Tomes-Oral capsules, the harvested serum was used, and MDA estimation was done via kit according to the manufacturer's protocol (Sigma Aldrich).

Estimation of GSH

GSH in pre-treated and post-treated groups of Tomes-Oral capsules was estimated by using the harvested serum, and GSH estimation was done via kit according to the manufacturer's protocol (Sigma Aldrich).

Enzyme Linked Immuno-sorbant assay (ELISA)

Solid phase sandwich ELISA was performed for **collagen-1 (skin tightening), VEGF (skin regeneration), glutathione reductase (GR), MMP-12 (elastase), and TNF- α (inflammation)** by the previously reported method with slight modifications (Wajid *et al.*, 2014). Briefly, a 96-well plate (Corning, USA) was coated with rabbit polyclonal anti VEGF, collagen-1, MMP-12, GR and TNF- α antibody (Santa Cruz Biotechnology, USA) and incubated for 120 minutes. After washing three times with tris buffered saline (TBS), blocking was done with 1% BSA. Once blocking was done, 100µl serum from all groups of treatment was loaded and incubated for 60 minutes. Samples were removed and rinsed wells three times with TBS-T and incubated overnight with HRP conjugated donkey anti rabbit secondary antibody (Santa Cruz biotechnology, USA) for 90 minutes. After washing, 100µl of chromogenic solution tetramethyl benzidine (TMB) (Invitrogen Inc., USA) was added and to stop the reaction 0.18M sulfuric acid was added. Absorbance was taken at a wavelength of 450nm.

Statistical analysis

All data of experimental groups was expressed as mean ± SEM in triplicate experiments. For statistical analysis, group means were compared by one-way ANOVA, and Bonferroni's test was used to identify differences between groups. Quantitative data obtained from different experimental groups was statistically analyzed via graph pad software by using two way ANOVA. P value less than 0.05 was considered as significant from statistical analysis. The level of significance of the p-value within a group was determined by asterisks (*/ **/***). One asterisk (*) indicated a low level of significance, and two asterisks (**) indicated a moderate level of significance. Three asterisks (***) indicated a high level of significance. Moreover, α and β also showed a significant level of the p-value between different groups. Specifications of the significant signs are given in the table below.

Intra group comparison denoted by *
Significant comparison of zero week untreated group with treatment and placebo groups of zero week
Significant comparison of 4 week untreated group with treatment and placebo groups of 4 week
Significant comparison of 8 week untreated group with treatment and placebo groups of 8 week
Inter group comparison denoted by α and β
α symbolized significant comparison of zero week treatment group (100mg/day) with treatment groups (100mg/day) of 4 and 8 weeks
β symbolized significant comparison of zero week treatment group (200mg/day) with treatment groups (200mg/day) of 4 and 8 weeks

5. Results

5.1 Assessment of TOMESORAL Intake on the vital biological functions of the Participants

For assessing the TOMESORAL (food supplement) and its different concentrations (*i.e.,* 250mg/day and 500mg/day) intake effect on the participant's vital biological functions, multiple parameters were examined in participants of all groups. These parameters include direct and total bilirubin assessment, alkaline phosphatase (ALP), vitamin D levels, fasting glucose level (FGL), and shock index (SI).

5.1.1 Serum Bilirubin Concentration

To evaluate the effect of TOMESORAL's two different concentrations on vital hepatic functions, direct and total serum bilirubin concentration were measured. Figures 1a and 1b show no significant difference between the normal and different treated groups. Only a slight variation in the basal levels was noted between the groups.

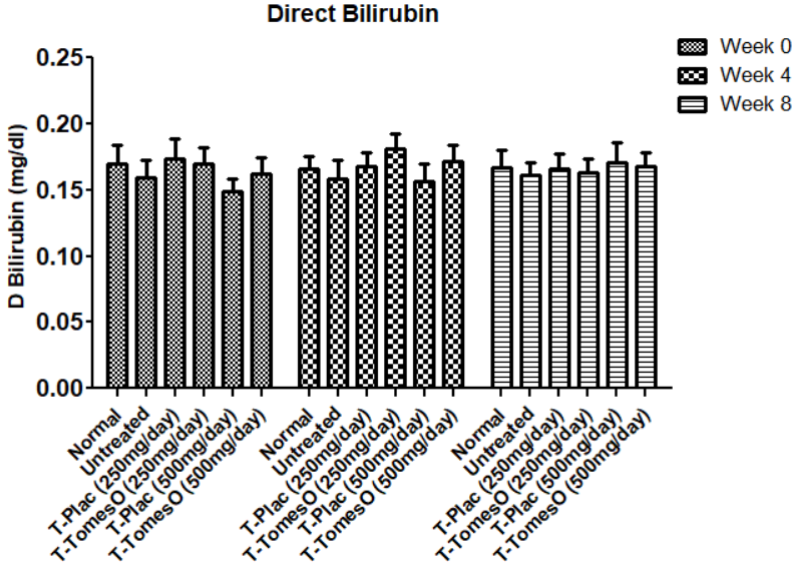

Figure 1a: Hepatic Vital Functions assessment: **Direct Bilirubin** was measured in all participants at day zero, 28, and 56. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day.

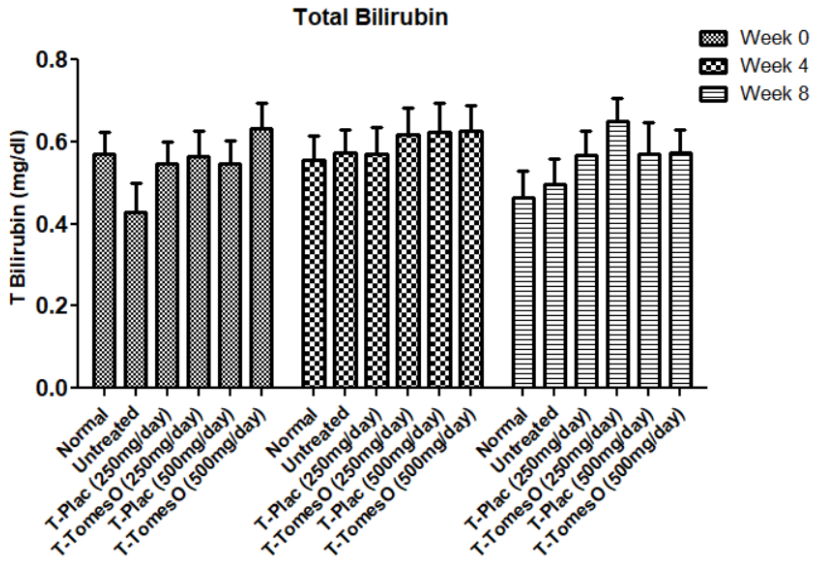

Figure 1b: Hepatic Vital Functions assessment: Total Bilirubin was checked in all participants at zero, 4th, and 8th week. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day.

5.1.2 Plasma Alkaline Phosphatase (ALP)

Plasma ALP values were examined in all healthy participant groups at 0, 4th and 8th week to investigate the effect of food supplement "TOMESORAL" consumption on vital hepatic functions. The graph in figure 1c indicates that ALP values in different participant groups were non-significantly different from the normal participant.

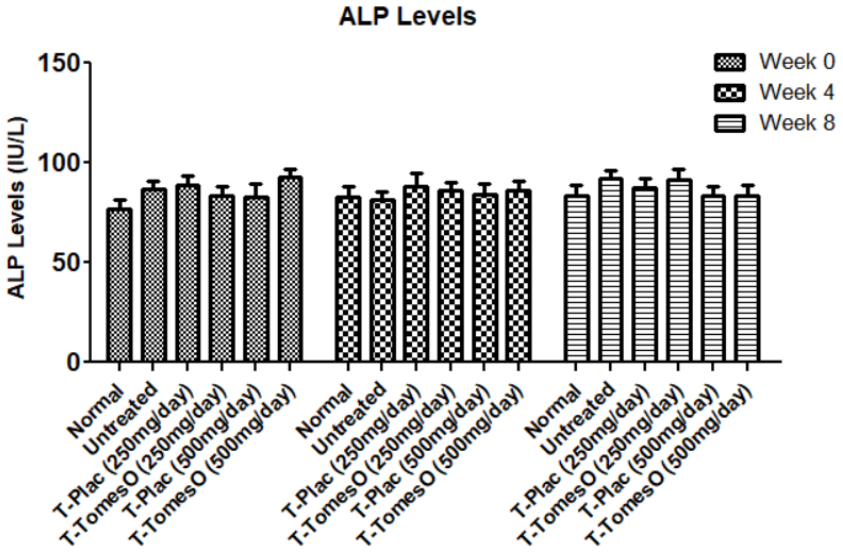

Figure 1c: Hepatic Vital Functions assessment: Plasma Alkaline phosphatase (ALP) values were assessed in all participants at days zero, 28, and 56. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day.

5.1.3. Vitamin D status in Participants

The average vitamin D status was determined to assess the effect of TOMESORAL on the vital functions of healthy participants. It was observed that the TOMESORAL intake did not significantly change the vitamin D status in all participants at zero, 4th, and 8th weeks. The levels of vitamin D in all group participants were similar to normal group participants (Figure 1d).

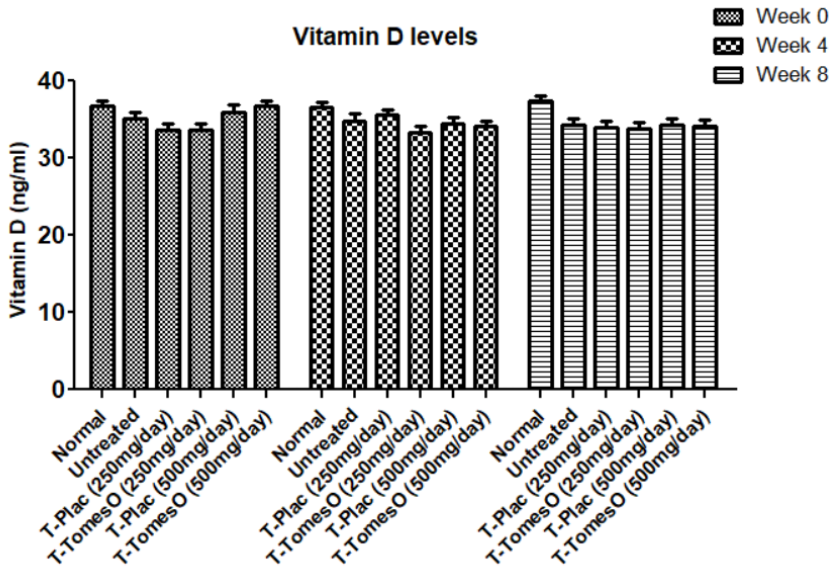

Figure 1d: Vital Biological Functions assessment: Vitamin D status was examined in all participants groups at 0, 4th, and 8th weeks. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day.

5.1.4 Fasting Glucose Levels (FGL)

FGL was another parameter among vital biological function assessment. It was noticed that the FGL remain the same among different participant groups at days zero, 28 and 56 (figure 1e).

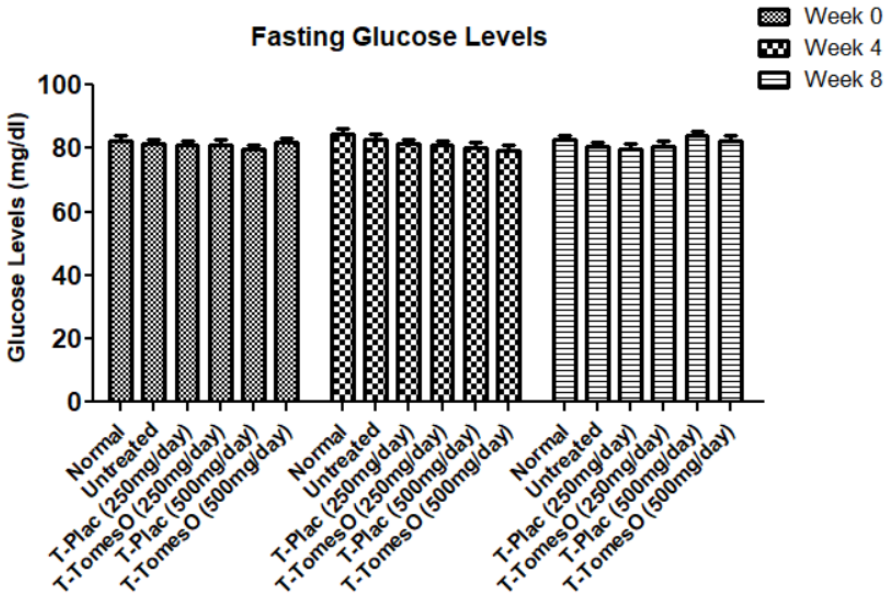

Figure 1e: Vital Biological Functions assessment: Fasting glucose levels (FGL) were checked in all participant groups at days 0, 28 and 56. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day.

5.1.5. Shock Index (SI)

SI is the ratio between the heart rate and systolic blood pressure (HR/SBP). It is a physiological score that can guide the initial emergency and prehospital care to evaluate the trauma severity and also to estimate an early hemorrhagic shock. SI index was also calculated to check the effect of TOMESORAL white supplement consumption effect on HR and SBP. The results revealed that the SI index remains unaffected post-TOMESORAL supplement intake and similar to the index of normal participants (figure 1f).

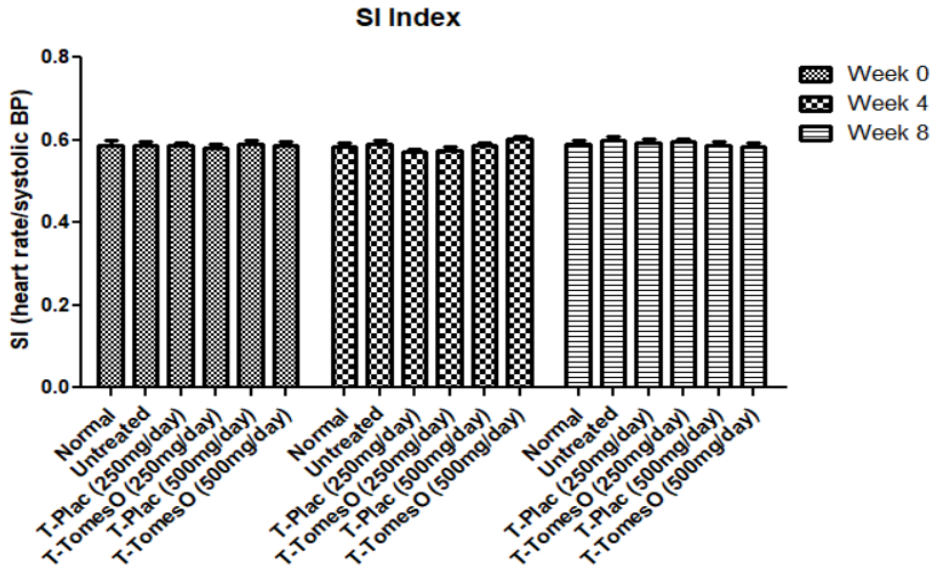

Figure 1f: Vital Biological Functions assessment: **Shock index (SI)** was calculated in all participant groups at 0, 4th and 8th. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day.

5.2. Effect of TOMESORAL food supplement on the skin stress

5.2.1. TOMESORAL curtails Lipid Peroxidation

Lipids oxidative damage is termed lipid peroxidation. Lipid peroxidation is caused by skin stress, such as from photo-damage. MDA levels were assessed in all participant groups at days zero, 28, and 56 for evaluation of the TOMESORAL effect on lipid peroxidation. The graph in figure 2 shows that in the untreated group post-56 days, MDA levels were significantly increased. Additionally, the graph also indicates that TOMESORAL at a dose of 500mg/day post-eight-weeks intake reduces the MDA level to a considerable extent compared to the 250mg/day dose of TOMESORAL. Moreover, the levels of MDA for T-Tomes O (500mg/day) group are nearly similar to that of normal group participants. Furthermore, MDA levels did not significantly alter after intake of TOMESORAL at a dose of 250mg/day post eight weeks. No effect of vehicle rice powder on MDA levels was observed.

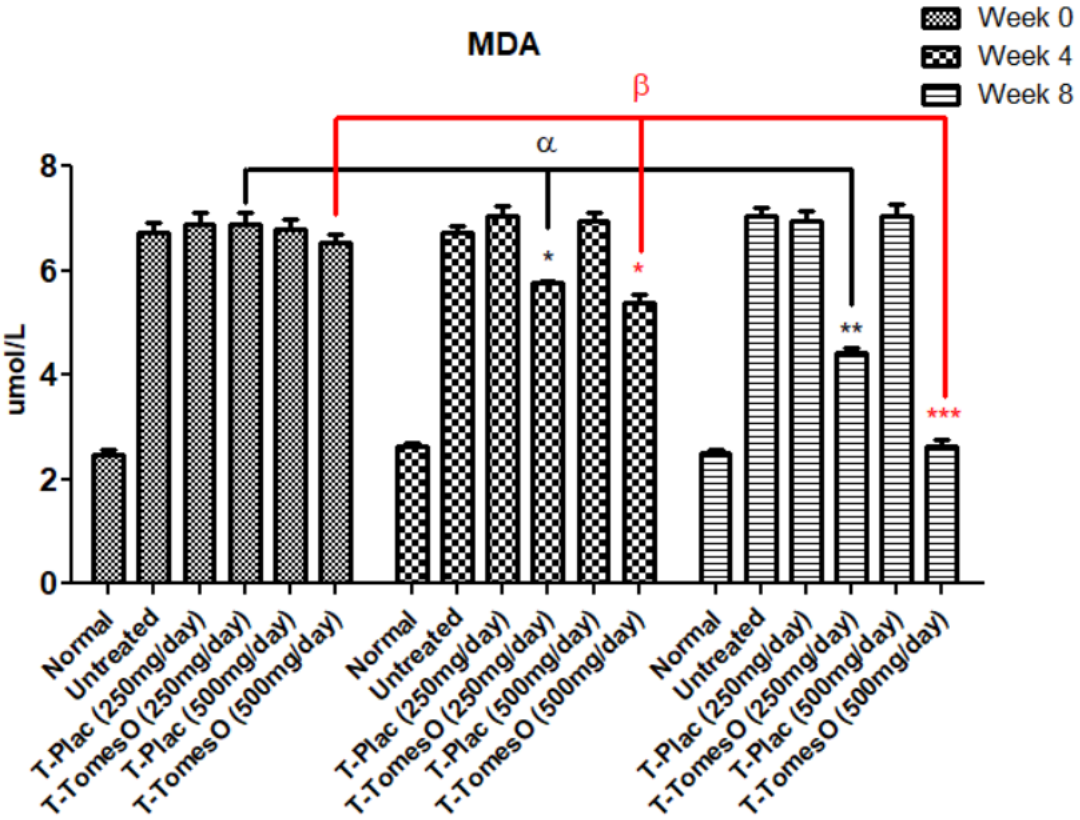

Figure 2: Clinical appraisal of Photo-damage Stress levels: MDA levels were assessed in all-participants groups at 0, 4, and 8 weeks to estimate the photo-damage stress level. The scale ranges from 0 to 8, where 0 indicates no stress and 8 indicates high-level stress. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.3 Effect of TOMESORAL food supplement on the Skin Lightening

5.3.1 TOMESORAL Augments Glutathione reductase (GR) and Reduced glutathione (GSH) levels

Glutathione reductase (GR) and reduced glutathione (GSH) levels were measured in all participants at days zero, 28 and 56 to evaluate the effect of TOMESORAL supplement on oxidative damage of the skin. Figures 3a and 3b show that remarkable improvement in GR and GSH levels was noticed after TOMESORAL intake at a dosage of 500mg/day for 56 days compared to TOMESORAL at a dose of 250mg/day and untreated groups. The levels of GR and GSH in T-Tomes O (500mg/ml) are similar to normal participants. Furthermore, it can be observed in graphs that rice powder has not affected skin oxidative damage.

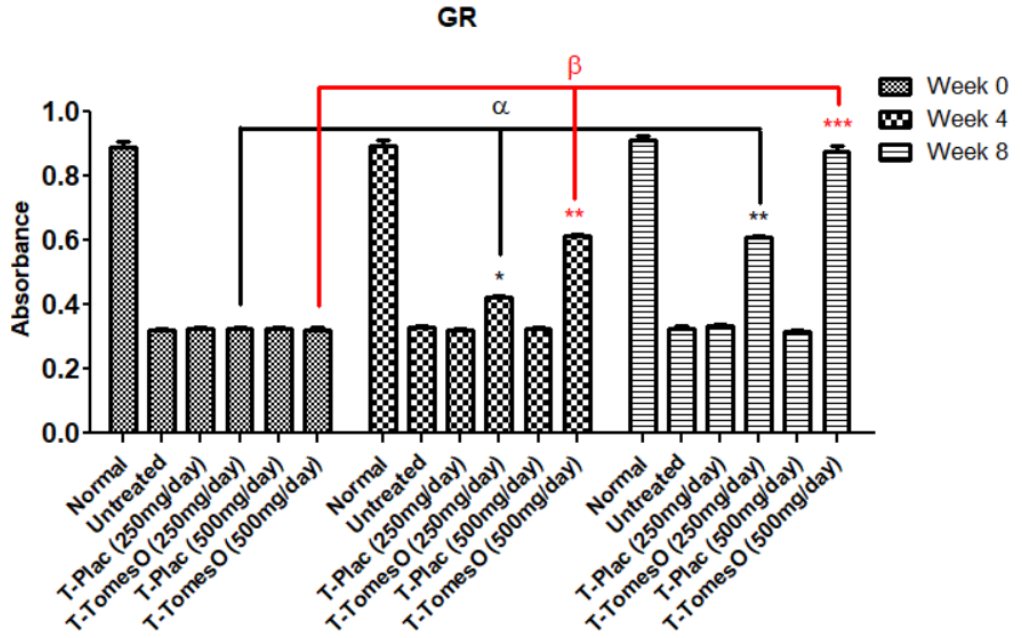

Figure 3a: Clinical appraisal of Oxidative damage: GR levels were measured in all groups at weeks zero, 4, and 8 to assess the oxidative damage. The scale ranges from 0 to 8, where 0 indicates no GR and 8 indicates high-level GR activity. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

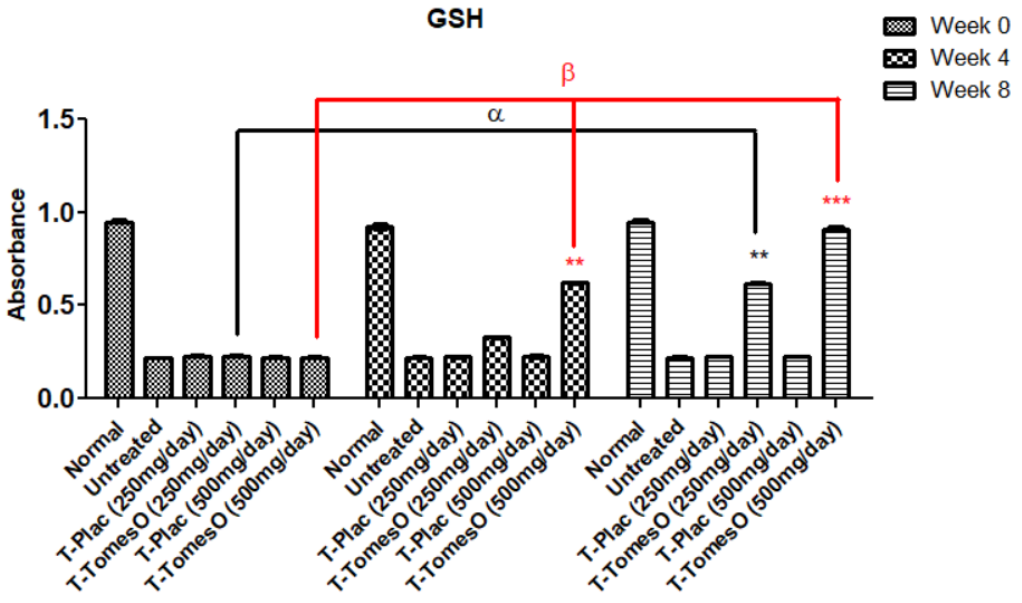

Figure 3b: Clinical appraisal of Antioxidants: GSH levels were measured in all groups at weeks zero, 4, and 8 to assess the oxidative damage. The scale ranges from 0 to 8, where 0 indicates no GSH and 8 indicates high-level GSH activity. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.3.2 Skin Lightening effects of TOMESORAL

Evaluation of lightening of skin tone in pre-treatment and post-treatment groups of Tomes O was done by quantification of pre-treatment and post-treatment face images with the help of Image J software. Figure 3c has shown a visible increase in lightened skin tone on the skin after 8-weeks intake of Tomes O at a dose of 500mg/day. The graphs in figure 3d indicates that, at 8th-week post-Tomes O oral intake 500mg/day, an apparent increase with a scale value of 23.21± 9.531 in skin tone was observed as compared to before treatment group with a scale value of 12.79± 5.626.

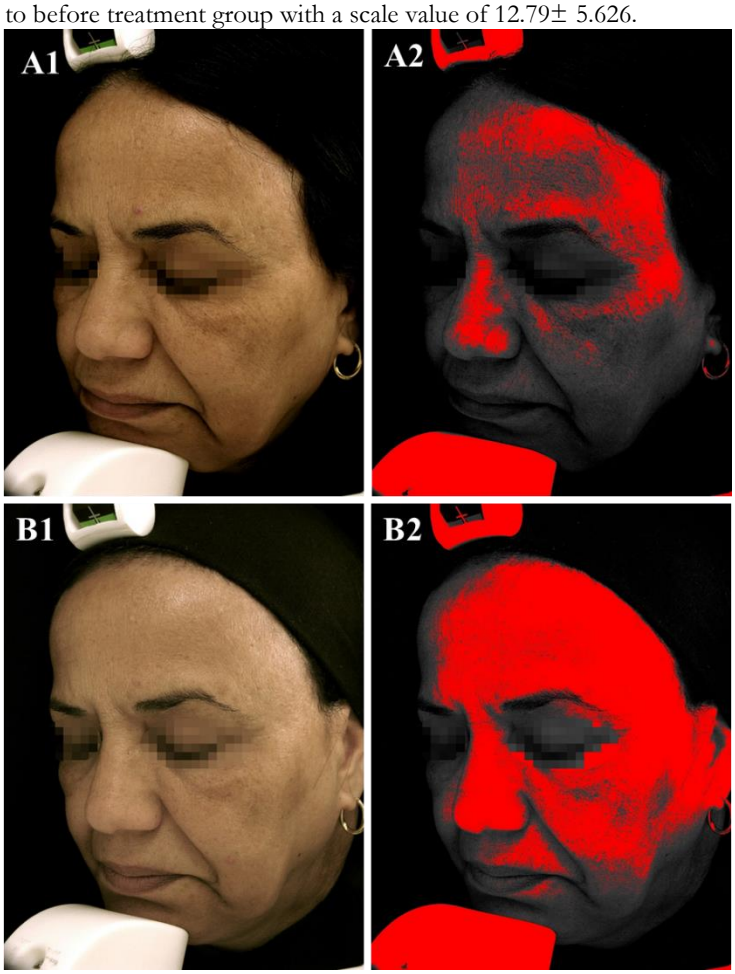

Figure 3c: Pictorial representation of the skin tone before (A1 and A2) and after treatment of TomesO supplement at eight weeks of oral intake (B1 and B2).

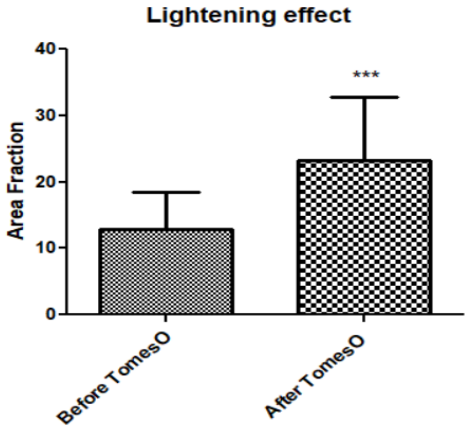

Figure 3d: Graphical representation of the skin tone before and after treatment of TomesO supplement at eight weeks of oral intake. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between before treatment group and after treatment group).

5.4. Damaged Skin Reconstruction

5.4.1. TOMESORAL food supplement improves Collagen I

To determine the effect of TOMESORAL food supplement on skin tightening, ELISA was used to measure the collagen I level. This assay results revealed that (figure 4a) collagen I level reduced remarkably in untreated groups after 56 days. While its levels significantly enhanced in the T-Tomes O (500mg/ml) group participants post-eight-weeks of consumption, which

was almost equal to that of normal group participants. In addition, compare to T-Tomes O (500mg/day) group, T-Tomes O (250mg/day) group did not cause a significant rise in collagen I levels. Vehicle rice powder has no effect on collagen I levels.

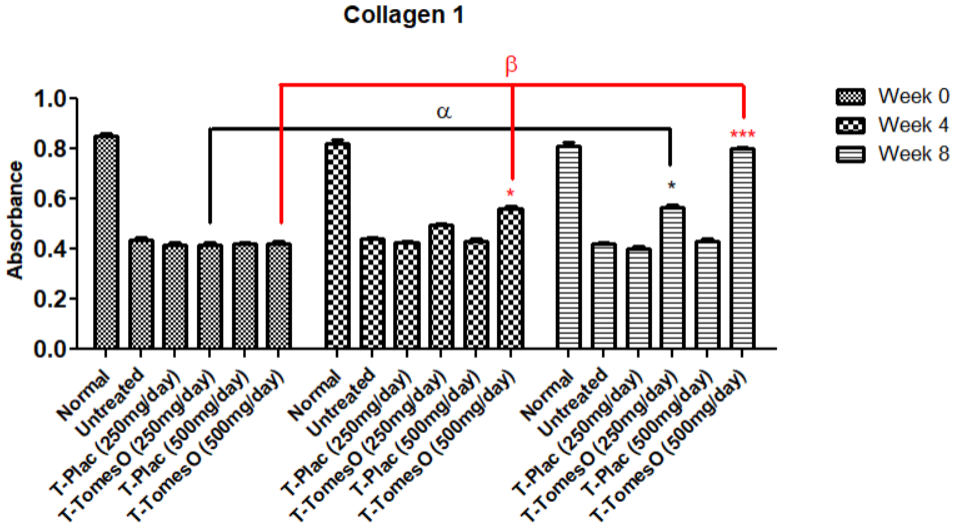

Figure 4a: Clinical appraisal of Skin tightening: Collagen I level was determined in all group participants at weeks 0, 4, and 8 to check the skin tightening. Scale ranges from 0 to 8, where 0 represents fewer collagen and 8 represents the highest collagen level. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.4.2. TomesO causes reduction of fine lines

Expert performed a clinical evaluation of the face and hands. At 0, 4 and 8 weeks, fine lines and wrinkles were assessed on the face of volunteers. Fine lines and wrinkles have been evaluated using the photo-numeric scale of Griffith. The scale is between 0 and 8, where 0 is without wrinkles, and 8 is the worst wrinkles. In figure 4b, graph indicates that a seeming reduction in the face fine lines and wrinkles at 8th week after TomesO (500mg/day) oral consumption (scale value of 3.66± 0.230) was observed in comparison with an untreated value of 6.86± 0.21. Placebo intake has no effect on volunteers' facial lines or wrinkles.

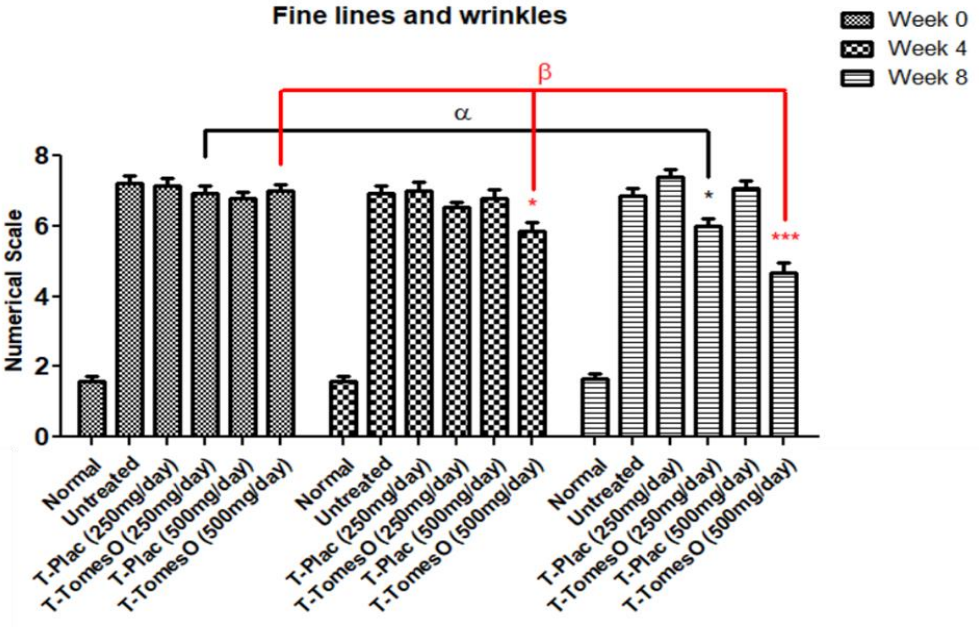

Figure 4b: Clinical assessment of skin fine lines and wrinkles: Skin fine lines and wrinkles measured in all volunteer groups at week zero, 4, and 8 according to Griffiths photonumeric scale. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the

significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.5. TOMESORAL food supplement increases Skin regeneration

5.5.1. TOMESORAL augments blood circulation by VEGF up-regulation

The effect of TOMESORAL on skin regeneration was analyzed by assessing the VEGF levels in all participants at days zero, 28, and 56. Figure 5a demonstrates that a significant decline in VEGF levels was observed in the untreated group. In contrast, a remarkable improvement in VEGF levels was seen in the T-TOMESORAL white group at a dose of 500mg/day post eight weeks. Additionally, 250mg/day dosage of TOMESORAL white for eight weeks was not effective in enhancing VEGF levels compared to TOMESORAL white at 500mg/ml. It was also noticed that VEGF levels were not changed by intake of rice powder.

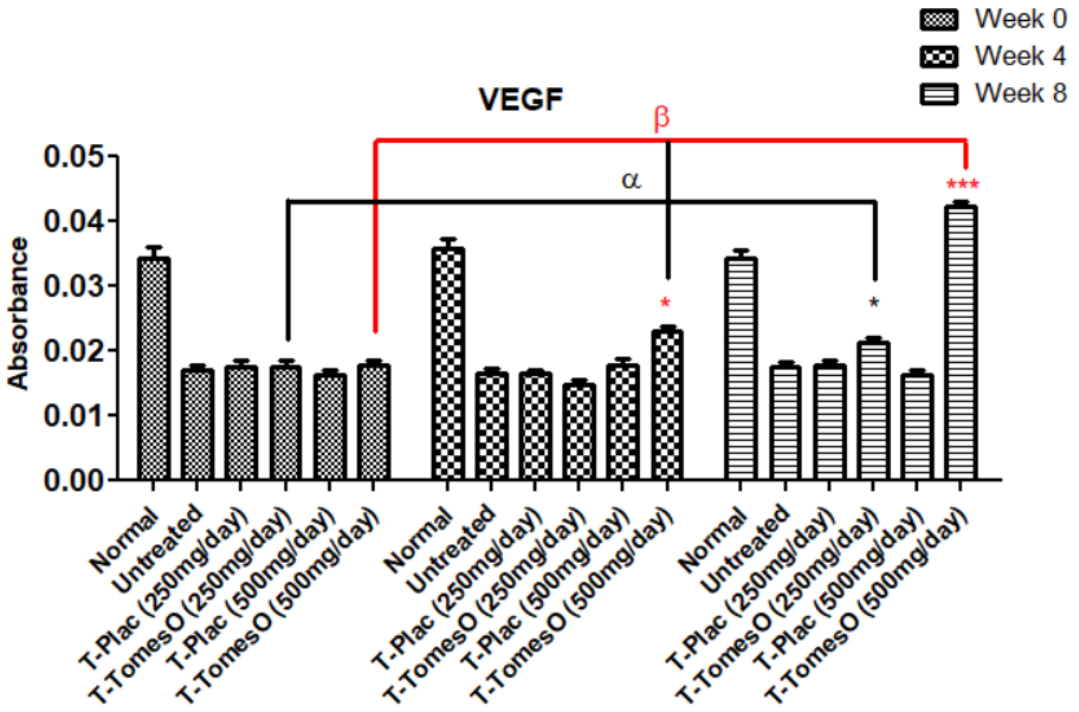

Figure 5a: Clinical appraisal of Skin regeneration: VEGF levels were evaluated in all participants group at week zero, 4, and 8 via ELISA to determine skin regeneration. The scale ranges from 0 to 8, where 0 indicates the least regeneration and 8 indicates an advanced level of skin regeneration. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.5.2. TOMESORAL supplement lessens the tactile roughness

Tactile roughness is the measure of skin smoothness with no rough patches. In this clinical assessment study, the TomesO effect was also assessed by scoring on the random areas from 0 to 8, where 0 represents smooth skin with no rough patches and 8 represents much-roughened skin. The results (figure 5b) revealed that a post-8-weeks intake of TomesO at oral dosing of 500mg/day caused a remarkable decline in tactile roughness with a score of 4.67±0.23 compared to untreated group with a score of 7.40±0.214. Compared to this, TomesO at an oral dose of 250mg/ml for eight weeks did not significantly reduce tactile roughness (5.9±0.19). Moreover, it was observed that placebo rice powder did not affect the outcome of tactile roughness in volunteers.

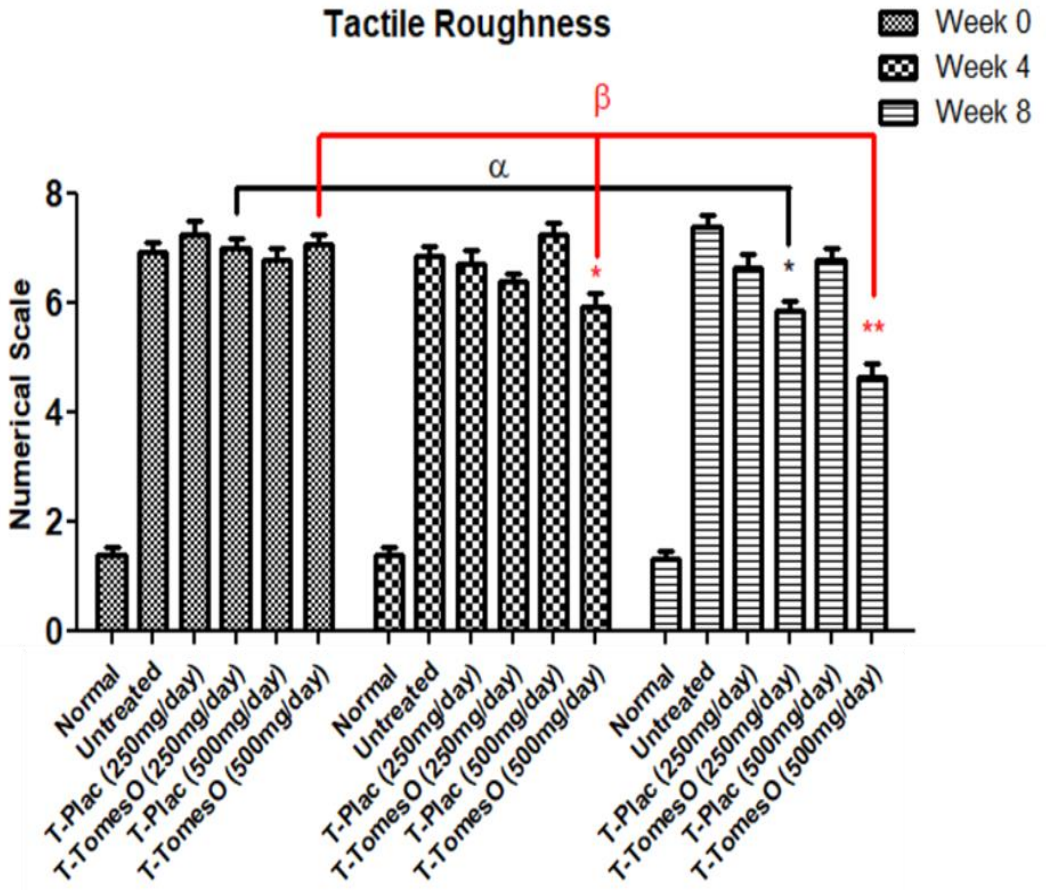

Figure 5b: Clinical assessment of tactile roughness: Tactile roughness checked in all volunteer groups at day zero, 28 and 56 according to Griffiths photonumeric scale. The scale ranges from 0 to 8, where 0 represents no tactile roughness, and 8 represents the most severe tactile roughness. Where normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.6. TOMESORAL food supplement reduces skin inflammation

5.6.1. Reduction in TNF-α Levels

TNF-α levels were measured via ELISA to evaluate the food supplement effect on inflammation of the skin. The graph (figure 7) reveals that eight weeks intake of TOMESORAL at500mg/day dose results in a significant decline in TNF-α expression levels compared to the untreated group, where an upsurge in levels can be seen. Additionally, 500mg/day dosage of TOMESORAL was more effective in decreasing the TNF-α levels than 250mg/day consumption of supplement for 56 days. While it can also be noticed that TNF-α levels in T-Tomes O (500mg/day) group are similar to that of normal group levels. No alteration was seen in TNF-α expression in vehicle rice powder group participants.

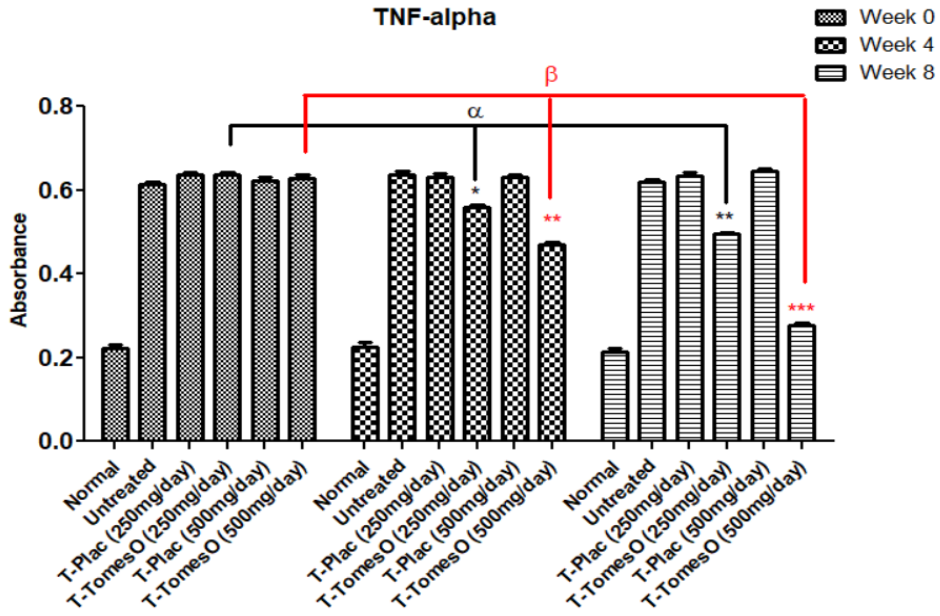

Figure 6a: Clinical appraisal of Skin inflammation: Tnf-α levels were determined in all subjects at weeks zero, 4, and 8 via ELISA assay. The scale ranges from 0 to 8, where 0 indicates no inflammation and 8 indicates high levels of skin inflammation. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

5.6.2. TOMESORAL food supplement alleviates MMP-12

Evaluation of the MMP-12 (elastase) enzyme was done in all participants at 0, 4 and 8 weeks to check the effect of a food supplement on this enzyme. The graph (figure 6) indicates that 500mg/day dosage of TOMESORAL for 8 weeks was more effective in alleviating MMP-12 as compared to TOMESORAL at 250mg/day dosage. Whereas enhanced MMP-12 levels were observed in the untreated group. Moreover, the graphs also demonstrate that the levels of MMP-12 in T-Tomes O (500mg/day) group are non-significantly different from the normal groups, while rice powder intake has no impact on MMP-12 levels.

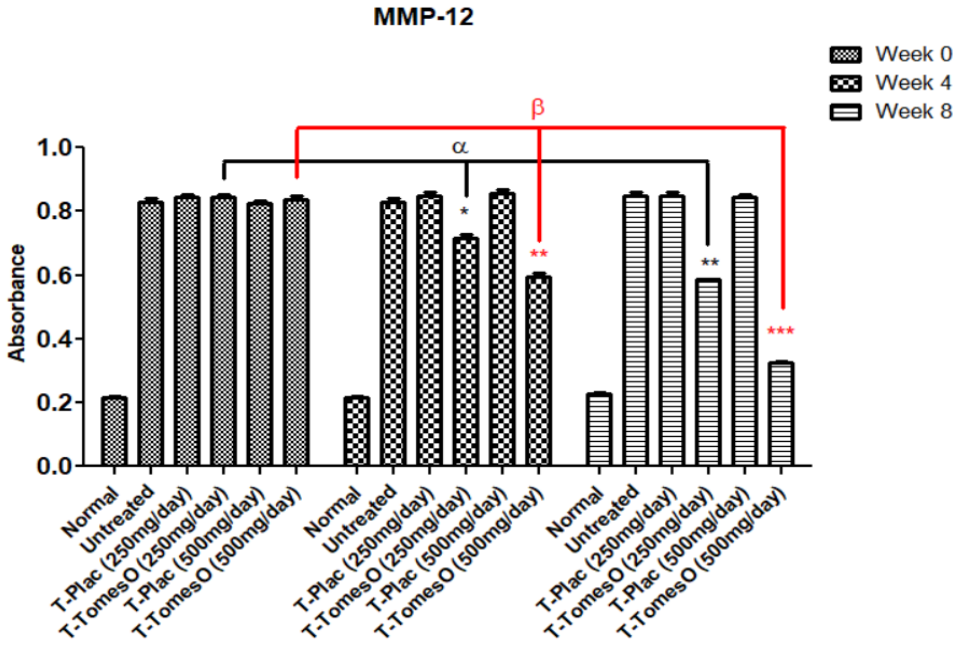

Figure 6b: Clinical appraisal of Collagenase-I: MMP-1 levels were assessed in all participants group at week zero, 4, and 8 via ELISA. The scale ranges from 0 to 8, where 0 indicates less MMP-1 and 8 indicates high-level MMP-1. The normal group indicates healthy persons with skin photo-type I and II, the untreated group includes persons with Skin Photo-type III and IV

with no treatment, T-Plac (250mg/day) group includes persons Treated with rice powder 250mg/day, T-Tomes O (250mg/day) includes participants treated with TOMESORAL 250mg/day, T-Plac (500mg/day) represents participants treated with rice powder 500mg/day and T-Tomes O (500mg/day) includes participants treated with TOMESORAL 500mg/day. Values were expressed as ±SEM. ($p<0.05$ consider as significant) (* shows the significant difference between treated groups and untreated control of same week) (α indicates the significance of 250mg/day groups of 4 and 8 weeks vs. zero week) (β indicates the significance of 500mg/day groups of 4 and 8 weeks vs. zero week).

DISCUSSION

The concept of photoprotection by dietary means is gaining attraction. Plant constituents such as carotenoids and flavonoids are involved in protection against excess light in plants and also contribute to the prevention of UV damage in humans skin (Stahl and Sies 2007). Ultraviolet (UV) rays are known to impact the skin externally (Afaq and K Katiyar 2011, Heinrich, Moore et al. 2011, Chen, Damian et al. 2014, Pérez-Sánchez, Barrajón-Catalán et al. 2014). Flavonoids form major constituents of the human diet as they contribute to the flavour and colour of many fruits and vegetables. Their beneficial antioxidant effects are thoroughly studied and established (Kiefer, Weibel et al. 2010). Carotenoids are valued for their protection against oxidative and free radical damage secondary to high-energy sources such as UV radiations. Available in topical and oral forms, the most abundant types include Phytoene, Phytofluene, lutein and zeaxanthin.

Tomatoes have been recognized as an important source of dietary flavonoids because of a high consumption worldwide. Heirloom tomatoes are varieties that have been grown without crossbreeding for 50 or more years. This is in contrast to the typical supermarket tomatoes, which are hybrids that have been carefully crossbred to have particular characteristics. TOMESORAL's properties provide a unique delivery system for better Bioavailability which are delivered at a cellular level for more intense effects and benefits and contain the active component needed for better absorption into the skin as well as stimulating collagen production, working synergistically with the supplement's other active ingredients to raise glutathione levels in the blood naturally. People over the age of 70 tend to "skin tear," because the skin is sensitive and easily broken (Xu, Lau et al. 2009). To prevent this problem skin elasticity is essential for good skin care from an early age.

Investigators recruit subjects with predetermined features during the trial, administer the treatment(s), and collect data for a given period of time on the health of the subjects. Data include measurements such as vital signs, measurement biochemical parameters in the serum samples of the TOMESORAL and associated groups, changes to symptoms, and improvement of the condition targeted by the TOMESORAL.

A calculation of the shock index (SI) (heart rate/systolic blood pressure; normal range, 0.5 to 0.7) and conventional vital signs for the identification of an acute or critical illness in pre-treatment and post-treated groups has been reported in many clinical trials (Rady, Smithline et al. 1994). The dose of the study drug or treatment is considered to be safe if both pre-treatment and post-treatment levels of SI and conventional vital signs fall in their normal ranges. The results of SI and vital signs for this present study also depict that there was no significant difference between these parameters of the pre-treatment and post-treatment groups.

This study provides evidence that use of TOMESORAL 'anti-ageing' nutraceutical product is able to induce clinically identifiable improvement in the appearance of fine lines and facial wrinkles following 8 week use. This improvement is associated with deposition of collagen-1 and decrease in elastase levels in the dermis of treated skin.

Some studies investigated that MMP-mediated collagen destruction accounts, in large part, for the connective tissue damage that results in dermal skin damage. In addition, the same group of investigators reported that type I and type III pro-collagen levels are significantly lowered in severely photo-damaged human skin. Thus, they claimed that collagen synthesis is reduced more in photo-aged human skin than in naturally aged skin in vivo (Chung, Seo et al. 2001). Recently, it has been suggested that collagen damage due to natural skin aging may arise, as it does in photo-aging, from elevated MMP expression with a concomitant reduction in collagen synthesis. Varani et al (Varani, Spearman et al. 2001) reported that with increasing age MMP levels become higher and collagen synthesis becomes lower in sun-protected human skin in vivo (Chung, Seo et al. 2001).

TNF-α and associated factors are known mediators of skin inflammation (Fleisher, Ferrell et al. 1990, Han, Tuan et al. 2001, Scott, Arnott et al. 2004). These markers are high in persons with photo-damaged skin while after the treatment of TOMESORAL their levels are significantly decreased showing the reduction in inflammation. This reduction in inflammatory markers and improvement in antioxidant shed numerous health benefits that result in vibrant skin texture as evident from TOMESORAL treatment group's results. Moreover, our result also show the increase in VEGF levels which resulted in better blood supply to skin by enhancing the angiogenesis and regenerate the skin.

Finally the present study concludes that the use of TOMESORAL for 8 weeks show significant improvement in skin antioxidant status, blood circulation, collagen and reduction in elastase (MMP12) with no harmful effects on vital signs of the volunteers.

Glutathione has recently become the most common "systemic skine-lighting agent" as a potent antioxidant with additional anti-melanogenic properties. Intravenous administration (IV) of glutathione was the ' most common ' and most controversial route for skin-lightning. Besides being one of the wealthiest antioxidants, it is produced as an antioxidant after its antimelanogenic properties have been identified (Exner, Wessner et al. 2000). It is important to know that glutathione exists in a reduced form (GSH) and an oxidized form (GSSG). The reduced form, GSH, seems to be instrumental in the depigmenting properties of this unique molecule. Apart from these two major forms, GSH may be esterified to form glutathione esters (Exner, Wessner et al. 2000). In the cell, glutathione reductase is responsible for maintaining the supply of reduced glutathione (GSH) (Couto, Wood et al. 2016). Whenever this enzyme got activated, it can convert oxidized glutathione produced as a result of oxidative stress to its reduced form that is beneficial to the cells. One of the very recent research recommended Highly Efficient Synthesis of reduced Glutathione via a Genetic Engineering Enzymatic Method to enhance

the effects of glutathione reductase (Huang and Yin 2020). TOMESORAL contribute in maintaining high levels of glutathione thus making the skin fairer and glowing.
Placebo group show no improvement in skin regeneration parameters while 500mg/day dose of TOMESORAL significantly reduces the fine line and wrinkles, regenerates the skin and improves the skin elasticity.

CONCLUSION

TOMESORAL treatment group expresses low levels lipid peroxidation and inflammation making TOMESORAL a potential option for treatment of inflammation due to photo-damage and improve the skin health. Moreover, the unique natural cocktail of carotenoids and flavonoids in TOMESORAL augments the increase of glutathione and glutathione reductase levels in photo-damaged skin that not only repair the damaged skin but also contribute to enhance the skin resistance against the damaging effects of UV radiations.

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