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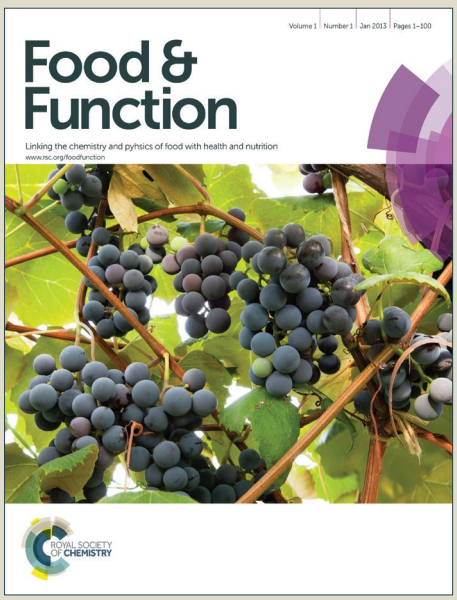

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Improvement of green tea polyphenol with milk on skin with respect to antioxidation in healthy adults: A double-blind placebo-controlled randomized crossover clinical trial

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Abstract

Green tea polyphenols (GTP) were widely tested for the several metabolic syndrome and degenerative diseases such as cancer, cardiovascular diseases, and diabetes. The present study was framed to assess the physiological efficacy of Green tea polyphenol infused with milk (GTPM) on skin integrity in correlation with antioxidative status in healthy adults. Forty-four healthy voluntary subjects were recruited and assigned to two groups,

who drunk 240 ml of mineral water mixed with either experimental (GTPM) or placebo package (2 packs/day) for the following 6 months and *vice versa* with one month of washout period in between. During the initial, 3rd, 6th, 10th, and 13th month anthropometric measurements were performed as well as fasting blood samples were withdrawn for various biochemical assays. Skin examination was performed at the initial, 6th and 13th month. No significant alterations were observed in any of the anthropometric measurements. Administration of GTPM significantly increased (p<0.05) the antioxidant index and antioxidant enzymes activities when compared with the placebo group, whereas a concomitant decrease in the levels of lipid peroxidation were noted. Moreover, GTPM intake notably improved skin integrity and texture by markedly lowered (p<0.05) skin wrinkles and roughness in elderly subjects. GTPM proved as an effective antioxidant by lowering oxidative stress and thereby ameliorate the skin texture and integrity.

Keyword: Green tea polyphenol, oxidative stress, antioxidation, lipid peroxidation

1. Introduction

Tea is second most famous beverage next to water. Green tea (non-fermented) and black tea (fully fermented) are the major types of tea. Black tea is consumed in Western countries that are rich in black tea polyphenols (BTP), i.e., theaflavins and thearubigins. Whereas, green tea is confined mainly to Asia and the Middle East and rich in green tea polyphenols (GTP) especially catechins.[1,2] Both BTP and GTP were widely tested for the several metabolic syndrome and degenerative diseases such as cancer, cardiovascular

diseases and diabetes.[3] Epidemiological studies indicates that GTP may exerts antioxidant, anti-inflammatory, antidiabetic, cardioprotective, anticarcinogenic, dermaprotective and antimutagenic effects ascribed to the presence of various types of catechins such as (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), and their epimerization isomers (+)-gallocatechin gallate (GCG), (+)-gallocatechin (GC), (+)-catechin gallate (CG), and (+)-catechin (C).[4-6] EGCG is a commonly studied tea polyphenol owing to its numerous beneficial properties.[7]

Despite, tea polyphenols are extensively consumed all over the world. Still, whether the addition of milk to tea, modulate the biological efficacy of tea polyphenols, especially catechins is still under debate. Several studies indicated that the addition of milk to tea might result in a reducing absorption of flavonoids (catechins) from the gastrointestinal tract due to the increased binding capacity of milk proteins (casein) with catechins of tea and thereby hindering their beneficial effects.[8,9] However, some studies proved that adding milk to tea had no effect on the plasma concentration of catechins and antioxidant status.[10-12] Dubeau and his colleagues[13] inferred that the addition of milk with tea had a dual effect on the antioxidant capacity. Few studies also demonstrate the consumption of tea with milk increases the plasma antioxidant potential in humans.[14,15] The above controversial results dragged our attention and propelled us to investigate the real issue between the tea polyphenol and milk protein for better understanding. Also till date, no long-term crossover clinical trials had been carried with green tea polyphenol and milk powder (GTPM) to assess the antioxidant capacity (beneficial effects) in healthy subjects.

Thus, the primary goal of the present work was to determine the antioxidant levels in healthy subjects (middle and old aged person) who consumed GTPM. As numerous clinical and preclinical studies showed that increased free radical production (oxidative stress) may probably end up with various dermal disorders, especially acne, psoriasis, porphyria's as well as early onset of aging.[16,17] Hence, the current intervention was designed to evaluate the long-term physiological efficacy of GTPM on skin texture via modulating antioxidant status in healthy adults.

2. Material and Methods

2.1 Chemicals

Folin–Ciocalteu phenol reagent, sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), hydrochloric acid (HCl), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), hydrogen peroxide (H_2O_2), sodium dihydrogen phosphate (NaH_2PO_4), and trichloroacetic acid (TCA) were purchased from Sigma (St. Louis, MO).

2.2 Determination of total phenolic and flavonoids contents in GTPM

Tea polyphenol milk samples were mixed with 10% acetonitrile in 10:1 ratio and homogenized for 30 min and filtered by using Whatman filter paper to get the extract as described by Lorenz method[9] with slight modification. The extract of GTPM were used to evaluate the total phenolic and flavonoids by the method of Julkunen-Titto and Wang.[18,19] The total phenolics and flavonoids contents were expressed as milligrams of gallic acid and quercetin equivalents per gram dry wt, respectively.

2.3 *In vivo* Studies (Clinical Trial)

2.3.1 Experimental (GTPM) and placebo

GTPM powder was provided by Standard Food Corporation, Taiwan. Each experimental packet (35 g) consisted of milk powder (Protein 6.5 g, Fat 3 g, Carbohydrate 22 g) with green tea polyphenols, skim milk, whey protein, lactoferrin isolated from soy and other supplements such as maltodextrin, vegetable oil, sugar, minerals (calcium carbonate, magnesium phosphate, zinc gluconate), vitamin (vitamin C, vitamin E, and folic acid), carrageenan. While the placebo pack also contains all the ingredients of the experimental package except for green tea polyphenols. Both the experimental and placebo packages were indistinguishably packaged with similar color, flavor, size and shape.

2.3.2 Subjects

This randomized, crossover, double-blind, placebo-controlled clinical trial was conducted at Chung Shan Medical University Hospital. The trial was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of Chung Shan Medical University Hospital, Taichung, Taiwan (Protocol No. CS08009). The volunteers (aged between 25 and 80) were recruited for the present intervention via advertisements. Written informed consent was obtained from all subjects before enrollment. The current crossover study, was planned with 44 participant based on expected dropout rate around

25% and thereby ensuring any lags or any serious impact on outcome (interpretation). Subjects were asked to abstain from consuming tea, tea-related products, vitamins, minerals, and dietary or herbal supplements during the intervention. If the subjects feel uncomfortable or distress with the intervention, they can withdraw at any juncture. Other exclusion criteria were heavy tea drinkers, history of smoking, alcoholism, pregnant or lactating women, hepatic or renal dysfunction.

2.3.3 Experimental Study

A physical examination was performed on 44 healthy voluntary subjects at the beginning of the study and they were segregated into two groups with 22 subjects in each. Each subjects was asked to consume 240 ml of mineral water mixed with either experimental or placebo package (2 packs/day at morning and evening with a minimum of 3 hours fasting) every day for the following 6 months and vice versa with one month of washout period in between. During the initial, 3^{rd}, 6^{th}, 10^{th}, and 13^{th} months anthropometric indices were done. Experimental (GTPM) or placebo package/bag were labeled with a subject number by the electrical randomization method. None of the researcher who treated or assessed the subjects was aware of the treatment allocation until the crossover study was completed (double blind). By the subject's record, the average percentage intake of experimental or placebo beverage was 84% at the conclusion of the experimentation. During the crossover study, two subjects were withdrawn from the study (due to personal reasons) and thus ended with 42 subjects (males 17, females 25).

2.3.4 Blood sampling

At the baseline (initial and 7th month), middle time point (3rd and 10th month) and end of each intervention period (6th and 13th month), fasting blood samples were collected in an EDTA coated vacuum tube and plasma was separated by centrifuging at 1500g (Supercentrifuge. 1K15. Sigma). Separated blood samples after deducting the intermediate film, settling part was washed with isotonic saline and centrifuged at 1500g to get erythrocytes for assaying antioxidant enzymes. All the samples were stored at −80 °C until analyzes.

2.3.5 Various oxidative indexes

Total antioxidant capacity of plasma was determined by Arnao[20] and Miller[21] with slightly modified. The total TBARS in plasma was determined by reacting with 2-thiobarbituric acid (TBA) at 90-100°C using Draper method.[22] GSH content in plasma was determined by the method of Halliwell and Gutteridge with slight modification.[23] The plasma ascorbic acid was determined by the method of Zannoni.[24] The total phenolic contents in plasma were determined by the method of Serafini.[25]

2.3.6 Antioxidative enzymes

Glutathione peroxidase (GPx), glutathione-S-Transferase (GST), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G-6-PDH) were determined by the Ransel RS 504, 125 kit (Randox Labs, Crumlin, UK) and catalase (CAT) was measured by the

method of Aebi.[26] Protein contents of RBCs were determined by using BCA kit from Thermo Fisher Scientific (Illinois, USA).

2.3.7. Skin examination

Multi-functional skin detector (MPA 580, Courage & Khazaka Electronic GmbH, Cologne, Germany) and skin roughness analyzer (VD 300, Courage & Khazaka Electronic GmbH, Cologne, Germany) were used to detect the biophysical characteristics of skin on face and arm region. Usage of any skin care products were strickly restricted prior to the measurements (24 h). Skin surface water loss, elasticity, erythema, melanin index, and hydration were measured with respective probes TEWAmeter, Cutometer Mexameter, and Corneometer (CK Electronic GmbH, Germany).

Skin hydration was measured by Corneometer CM 825, which use the high dielectric constant of water for determining the water-related changes in the electrical capacitance and hydration were measurements in system-specific arbitrary units.[27] Melanin index was measured by Mexameter MX 18 based on the strength of the absorbed and the reflected light at 660 nm and 880 nm, respectively. An erythema index is processed similarly at 568 nm and 660 nm, respectively.[28] The levels of TEWL were measured by TEWAmeter TM 300 and expressed as $g/m^2/h$.[29] Cutometer MPA 580 pulls the skin into the probe by controlled vacuum pressure.[30] Skin wrinkles, elasticity, and roughness, were measured using Visioscan VC 98 (Courage & Khazaka, Cologne, Germany) by using skin picture (texture). In brief, two points were measured, one was the middle of the nose and right

ear, the other one was the middle of wrist and elbow joint of the arm inner side. All subjects were requested to wash and clean their face and arm, and then stayed calmly for 30 minutes in a 20 ± 2 ˚C and RH 50 ± 2 % room before the test.

2.4 Statistical analysis

The results were expressed as a mean ± standard deviation (S.D). The paired t-test was employed to compare the difference in the same group (baseline vs. end of treatment), and the Student's t-test was used to compare the difference between the experimental (GTPM) and control (placebo) groups and the variables were analyzed via one-way ANOVA with post hoc LSD test. $p < 0.05$ was considered statistically significant. Statistical Package for the Social Sciences (SPSS) version 17.0 (Chicago, USA) was used for analysis.

3. Results and Discussion

Tea is the most popular beverage and second only to water, owing to its various bioactive compounds such as flavonoids, tannins, gallic acid and other phenolic acids.[31] The endeavor of the present work was to determine the physiological efficacy of GTPM on dermal changes with relationship to anti-oxidation in healthy adults. A schematic representation of the present study was illustrated in figure 1. Polyphenols contain one or more aromatic ring with several free hyroxyl group that make them as a potent antioxidant, which has been proven in numerous *in vitro* studies and clinical trials. Table

1 represented the total phenolics and flavonoids contents in GTPM package. The total phenolic contents estimated by Folin–Ciocalteu assay of GTPM displayed the value of 131.37 ± 9.2 mg GAE/g dry weight, whereas in case of the flavonoids content of GTPM showed 91.70 ± 0.5 mg QE/g dry weight. These results showed that both the phenolic and flavonoids contents were rich in GTPM package when compared with placebo (data not shown), those polyphenols were absent in the placebo group.

The anthropometric parameters were presented in table 2. No substantial alterations were marked on body weight, fat and body mass index (BMI) during the initial phase (baseline) of placebo and GTPM group. Even at the end of the intervention (after 6 months) no marked changes in the levels of body weight, fat and body mass index (BMI) as compared to baseline values of either group. Moreover, no concomitant variation was noted between placebo vs. GTPM groups in any of the anthropometric indices at the end of the experiment. The total phenolic contents in plasma of GTPM and placebo-treated healthy subjects were represented in table 3. Administration of GTPM showed a substantial enhancement ($p<0.05$) in total phenolics contents (3.51 ± 0.16 μg/mL to 3.75 ± 0.10 μg/mL) were noticed on comparison between baseline and 6th month. A significant difference was found between placebo and GTPM group on the 6th month. GTPM exhibits positive modulation over phenolic contents due to increased phenolic contents in GTPM package, which were indicated in table 1. This result are well correlated with that of Cabrera et al.[31] who demonstrated the addition of milk to tea did not interfere with catechin absorption instead it improved its bioavailability. Numerous studies show increasing intake of GTP eventually elevate the plasma phenolic contents.[10,32]

Antioxidants are molecule, that can interact with oxidant and thereby terminate free radicals generation. Various plasma oxidative indexes (TEAC, TBARS, GSH, Vit-c) in placebo and GTPM treated healthy subjects were epitomized in table 3. Drinking GTPM infusion for six months markedly improved (p<0.05) the levels of TEAC (0.54 to 0.59 μM), GSH (18.08 to 23.90 μM) and Vit-c (1.12 to 1.40 μM) whereas the TBARS (1.00 to 0.63 μM) levels were significantly suppressed (p<0.05) when compared with a baseline period of GTPM. A marked variation was noted between the GTPM and the placebo group on the 6th month. As discussed earlier, increasing plasma phenolic contents probably regulated those oxidative indexes in a positive way, and thus lowered the free radical generation and subsequently decreased the TBARS levels. Coimbra and his collagues[34] had evaluated the effect of green tea against oxidative stress in aged subjects and found that GTP can effectively lower the TBARS levels in RBC membrane. Moreover, adding milk to green tea decreases the formation of hydrogen peroxide (form of free radical) by forming a complex (catechin-casein), which breakdown hydrogen peroxide into oxygen and water.[32,34]

The antioxidant in human body neutralize free radicals, these antioxidants are called endogenous ones. The major endogenous antioxidants CAT, GPx, GST, and GR. CAT and GPx both convert hydrogen peroxide to water and oxygen by the expense of GSH, the GR-catalyzed regeneration of GSH from its oxidized form can sustain the GSH-dependent oxy-radical scavenging activity.[35] The antioxidant enzymes such as CAT, GPx, GST, GR and G-6-PDH in the erythrocyte of placebo and GTPM treated healthy

subjects were epitomized in table 4. Administration of GTPM exhibited a healthy enhancement in the levels of CAT (214.49 to 351.68 IU/g Hb), GPx (49.96 to 55.04 IU/g Hb), GST (4.85 to 8.74 IU/g Hb), GR (9.16 to 11.86 IU/g Hb) and G-6-PDH (1490.94 to 1754.61 IU/g Hb) on a comparison between baseline and the end of the treatment (6^{th} month). A notable alteration was also observed between GTPM and a placebo group on the 6^{th} month. The main contributor to the antioxidant activity in GTPM could be the catechins especially EGCG, which had been reported to activate nuclear factor erythroid 2-related factor 2 (Nrf2) which in turn up-regulating the downstream antioxidant genes and thereby by enhancing antioxidant enzymes levels.[36,37]

Several studies suggest that the addition of milk to tea might modulate its biological efficacy (decrease, increase or no effect) by reducing the absorption of flavonoids (catechins) from the gastrointestinal tract due to the increased binding capacity of milk proteins (casein) with catechins of tea.[8,9] Few studies prove that adding milk to tea had no effect on the plasma concentration of catechins and antioxidant status.[10-12] Dubeau and his colleagues[13] inferred that the addition of milk with tea had a dual effect on the antioxidant capacity. However, some studies also demonstrate the consumption of tea with milk increases in plasma antioxidant potential in humans.[14,15] Furthermore, Xie et al.[38] showed that milk can enhance the intestinal absorption of green tea catechins (EGCG) in Caco-2 cell model.

It has been reported that the non-gallated catechins (EGC, EC) are more stable than gallated derivatives (EGCG, ECG) during digestion process, which may contribute to

lower bioavailability of EGCG.[39,40] Moreover the free hydroxyl (OH) group in catechin (EGCG) makes it more hydrophilic in nature and leads to lower bioavailability of EGCG.[41] Hence, by infusion of milk (casein) with GTP probably favor the gallated catechin more stable by forming a complex (catechin-casein), which in turn improves the intestinal transport rate[38] as well as mask the free hydroxyl group and thereby increasing the bioavailability of EGCG. Moreover, scientific evidence supports the beneficial effects of GTP consumption and closely relates to catechins especially EGCG.[42] Probably the increased antioxidant activity in GTPM group might be due to increased bioavailability of EGCG, which has been confirmed by elevated phenolic contents in plasma (table 4). Those results are in agreement with Moser and his coworkers,[43] indicated that bioaccessibility (bioavailability) of catechins were improved by the infusion of GTP with milk proteins. Henning et al.[44] also showed that antioxidant activity of plasma was increased in GTP due to enhanced bioavailability. Bourassa et al.[45] pointed out that EGC had improved its antioxidant capacity, while binding with milk protein (casein) through pyrogallol group, where the active formation of free radicals are generated. Therefore, by forbidding the pyrogallol group of EGC the free radical generation was substantially lowered, which could be the reason for enhanced the antioxidant activity. Calcium and magnesium present in the milk and mineral water of GTPM favor the metallic-polyphenol complexes, which has been previously proved.[46] Furthermore, such complexes were reported to be less susceptible to oxidation when compared with uncomplexed catechins.[47]

Human skin acts as a barrier between the internal and the external environment, protecting the body from mechanical damage, noxious substances, and penetration by pathogens, radiation as well as regulated excretion of metabolic waste products and maintain temperature.[48] The skin is one of the major targets of free radical attack since it is directly exposed to UV radiation and a variety of environmental pollutants and in addition, they are rich in polyunsaturated fatty acids, that are highly susceptible for oxidation. Numerous pre-clinical and clinical studies showed that increased oxidative stress may probably end with various dermal disorders especially acne, psoriasis, porphyria's as well as early onset of aging.[16,17] Hence, the study was blueprinted to determine the long-term physiological efficacy of GTPM on skin texture via modulating antioxidant status in healthy adults.

Multifunctional skin analyzer (MPA 580- Courage & Khazaka Electronic GmbH, Cologne, Germany) was used to detect the skin changes in face and arm region. The skin parameters including skin elasticity, moisture, surface water loss, melanin, erythema and skin wrinkles were analyzed. Table 5 represented the skin examination in placebo and GTPM treated healthy subjects. In the placebo group, no marked changes were observed in any of the skin parameters. Whereas on infusion with GTPM subjects for 6 months exhibited no significant alterations in neither the face nor arm region in related to any of the skin parameters on compared with baseline. However, slight variation (but not significant) were noticed in skin elasticity and wrinkles, which influenced us to concentrate on aged subjects alone, as their antioxidant levels were less on compared to

middle-aged subjects. During the aging process, the oxidative damages are mostly found in parallel with the declined capacities of antioxidant systems.[49]

Table 6 showed the skin examination in placebo and GTPM treated subjects over 60 years old. Consumption of GTPM substantially augmented the levels of skin elasticity but concomitantly attenuated the levels of skin wrinkles also a slight decrease in the levels of skin roughness was found. No significant alteration was seen in the erythema index in both groups. These results signified the importance of GTPM on aging process too. Enhanced antioxidant might improve the skin integrity and texture in older subjects by increasing skin elasticity with decreased wrinkle and roughness. GTP has been reported to have some positive impact on cellular and molecular level in the epidermis and thereby act as a derma protective agent.[50] Moreover, ascorbic acid have been reported to lower oxidative stress by acting as a skin barrier.[51] As discussed earlier during the intake of GTPM, ascorbic acid levels were significantly elevated and thus ended up with enhancing epidermal differentiation, but more effective in aged subjects owing to elevated oxidative stress. It is well documented that UV light can initiate free radical in the epidermis by inducing lipid oxidation, which results in premature aging of the skin.[52] Oral administration of GTP (EGCG) significantly lowers the oxidative stress initiated by UV light in the skin.[53,54] Furthermore, EGCG markedly reduces UVB-induced MMP-1, MMP-8, and MMP-13 in a dose-dependent manner via its interference with mitogen-activated protein kinase-responsive pathways.[55]

Figure 2 depicted the skin surface topography of subjects 13 viewed under ultraviolet light. Figure 2A and C represents the facial and arm skin of placebo group, which indicated less skin integrity, whereas figure 2B and D represented the facial and arm skin of 6 months treated with GTPM, which indicate improved skin integrity and texture. Elevated free radicals are responsible for the weakening of elastin and collagen hence ends up in increased skin wrinkles, fragility, dull appearance and aging.[56] Due to derma protective and antioxidant activity of GTP and improved bioavailability of catechins (EGCG) by milk powder, this made GTPM a perfect blend to exhibit its holistic effect by improving skin elasticity and texture. Limitation of the present study was usage of less number of subjects, no separate group for GTP alone to compare with GTPM and the bioavailability of catechins in plasma were not carried out as well as absorption of catechins was affected by various factors. As it is a pilot study, we initiated with few numbers of subjects and a randomized placebo cross-over study, which restricted us to add more grouping for the present study. In recent time, we started to work on kinetics studies, which could give a detailed mechanism for the metabolism of GTPM.

4. Conclusion

Controversy to most of the research work, the present long-term crossover study displayed an elevation in the levels of phenolic contents (catechin) in plasma of GTPM group, it might be due to increased bioavailability of EGCG by forming a complex with milk protein (casein) and minerals and thus augmented the antioxidant status by lowering oxidative stress, which eventually ended up with improved skin integrity and texture

especially in old aged subjects. These findings will contribute to the development of novel derma protective agent, but few more studies are required to strengthen the beneficial effect of GTPM.

Author Contributions

HFC, YCS, KV and CKW designed and conceived the study protocol. HFC, YCS, and TYL helped in conducting the clinical trial. CKW and KV collected data for stastically analysis, as well as wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Chung Shan Medical University Hospital, Taichung, Taiwan (CS 08009) for funding this intervention.

References

1. J. Ye, F. Fan, X. Xu, and Y. Liang, *Food Res Int*, 2013, **53(1)**, 449-455.
2. S. K. Bøhn, K. D. Croft, S. Burrows, I. B. Puddey, T. P. Mulder, D. Fuchs, R. J. Woodman and J. M. Hodgson, *Food Funct*, 2014, **5**, 1613-1620.
3. J. V. Higdon, and B. Frei, *Crit Rev Food Sci Nutr*, 2003, **43**, 89-143.
4. K. Nakachi, S. Matsuyama, S. Miyake, M. Suganuma and K. Imai, *Biofactors*, 2000, **13**, 49-54.
5. J. Sano, S. Inami, K. Seimiya, T. Ohba, S. Sakai, T. Takano and K. Mizuno, *Circulation Journal*, 2004, **68**, 665-670.
6. C. Dufresne and E. Farnworth, *Food Res Int*, 2000, **33**, 409-421.

7. A. Guri, Y. Li and M. Corredig, *Food Funct*, 2015. DOI: 10.1039/c5fo00654f
8. S. Egert, J. Tereszczuk, S. Wein, M. Müller, J. Frank, G. Rimbach and S. Wolffram, *Eur J Nutr*, 2013, **52**, 281-288.
9. M. Lorenz, N. Jochmann, A. von Krosigk, P. Martus, G. Baumann, K. Stangl and V. Stangl, *Eur Heart J*, 2007, **28**, 219-223.
10. R. Leenen, A. Roodenburg, L. Tijburg and S. Wiseman, *Eur J Clin Nut*, 2000, **54**, 87-92.
11. P. C. Hollman, K. H. Van Het Hof, L. B. Tijburg and M. B. Katan, *Free Radic Res*, 2001, **34**, 297-300.
12. V. C. Reddy, G. Vidya Sagar, D. Sreeramulu, L. Venu and M. Raghunath, *Ann Nutr Metab*, 2005, **49**, 189-195.
13. S. Dubeau, G. Samson and H.-A. Tajmir-Riahi, *Food chem*, 2010, **122**, 539-545.
14. S. C. Langley-Evans, *Int J Food Sci Nutr*, 2000, **51**, 309-315.
15. V. Sharma, H. V. Kumar and L. J. M. Rao, *Food Res Int*, 2008, **41**, 124-129.
16. J. Kruk and E. Duchnik, *Asian Pac J Cancer Prev*, 2014, **15**, 561-568.
17. M. C. Velarde, J. M. Flynn, N. U. Day, S. Melov and J. Campisi, *Aging (Albany NY)*, 2012, **4**, 3.
18. R. Julkunen-Tiitto, *J Agric Food Chem*, 1985, **33**, 213-217.
19. C. Wang and L. Hwang, *J Chin Agric Chem Soc*, 1993, **31**, 623-623.
20. M. Arnao, J. Casas, J. Del Rio, M. Acosta and F. Garcia-Canovas, *Anal Biochem*, 1990, **185**, 335-338.
21. N. J. Miller, C. Rice-Evans, M. J. Davies, V. Gopinathan, and A. Milner, *Clin Sci*, 1993, **84**, 407-412.
22. H. Draper and M. Hadley, *Methods Enzymol*, 1990, **186**, 421-431.
23. B. Halliwell, and J. M. Gutteridge, *Arch Biochem Biophys,* 1990, **280**, 1-8.
24. V. Zannoni, M. Lynch, S. Goldstein and P. Sato, *Biochem Med*, 1974, **11**, 41-48.
25. M. Serafini, A. Ghiselli, and A Ferro-Luzzi, *Eur J Clin Nutr*, 1996, **50**, 28-32.
26. H. Aebi, *Methods Enzymol,* 1984, **105**, 121-126.
27. L. C, Gerhardt, V. Strässle, A. Lenz, N. D. Spencer, and S, Derler, *J Royal Society Interface,* 2008, **5(28)**, 1317-1328.

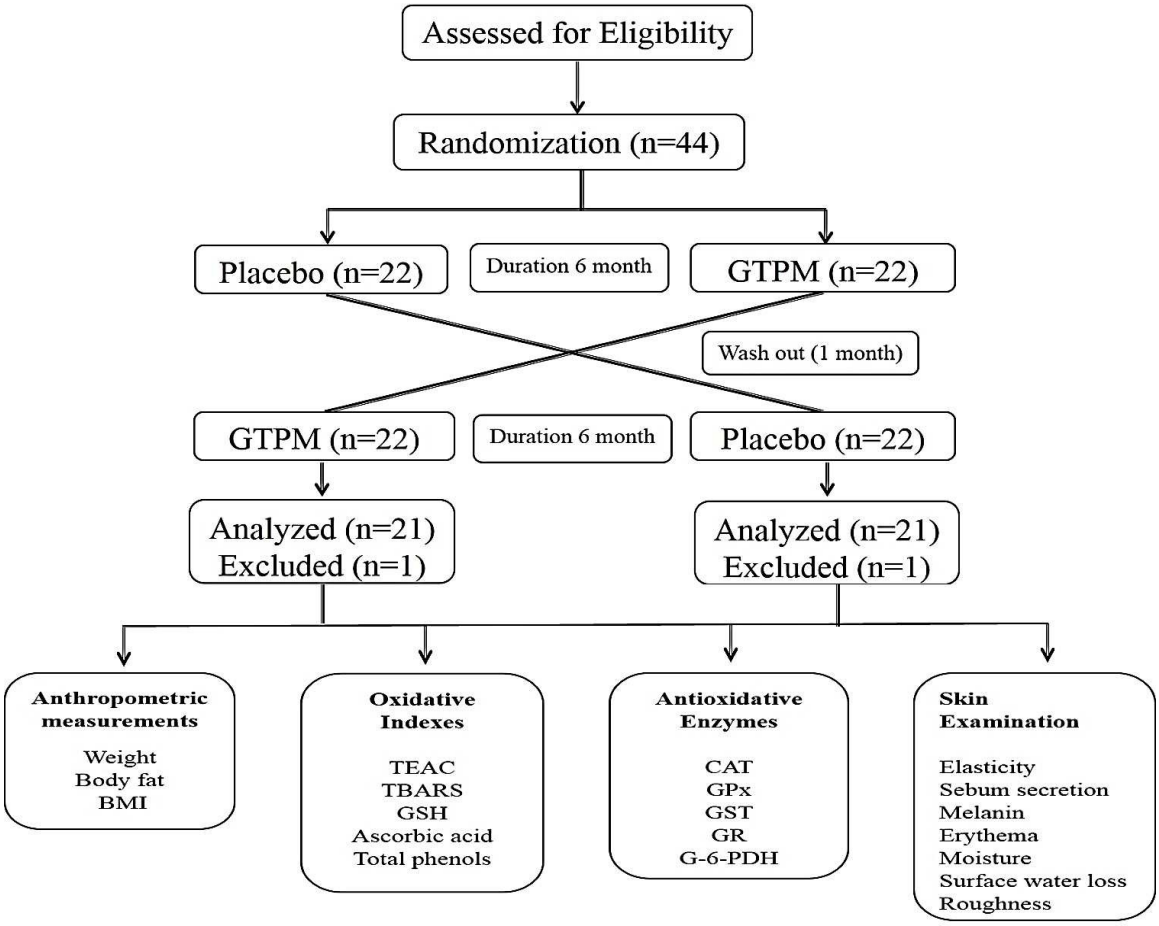
28. T. Yamamoto, H. Takiwaki, S. Arase, H. Ohshima, *Skin Res Tech,* 2008, **14(1)**, 26-34.
29. J. H. Shah, H. Zhai, and H. I. Maibach, *Skin Res Tech,* 2005, **11(3)**, 205-208.
30. H. S Ryu, Y. H. Joo, S. O. Kim, K. C. Park, S. W. Youn, *Skin Res Tech,* 2008, **14(3)**, 354-358.
31. C. Cabrera, R. Artacho and R. Giménez, *J Am Coll Nutr*, 2006, **25**, 79-99.
32. S. C. Forester and J. D. Lambert, *Mol Nutr Food Res,* 2011, **55**, 844-854.
33. S. Coimbra, E. Castro, P. Rocha-Pereira, I. Rebelo, S. Rocha and A. Santos-Silva, *Clin Nutr*, 2006, **25**, 790-796
34. L. H. Long, A. N. B. Lan, F. T. Y. Hsuan and B. Halliwell, *Free Radic Res*, 1999, **31**, 67-71.
35. I. Khan, A. M. Yousif, S. K. Johnson and S. Gamlath, *Clin Nutr*, 2015, **34**, 415-421.
36. J. Han, M. Wang, X. Jing, H. Shi, M. Ren and H. Lou, *Neurochem Res*, 2014, **39**, 1292-1299.
37. H.-K. Na and Y.-J. Surh, *Food Chem Toxicol*, 2008, **46**, 1271-1278.
38. Y. Xie, A. Kosińska, H. Xu and W. Andlauer, *Food Res Int*, 2013, **53**, 793-800.
39. R. J. Green, A. S. Murphy, B. Schulz, B. A. Watkins and M. G. Ferruzzi, *Mol Nut Food Res*, 2007, **51**, 1152-1162.
40. A. Marchese, E. Coppo, A. P. Sobolev, D. Rossi, L. Mannina and M. Daglia, *Food Res Int*, 2014, **63**, 182-191.
41. C. Auger, W. Mullen, Y. Hara and A. Crozier, *J Nutr*, 2008, **138**, 1535S-1542S.
42. S. M. Henning, C. Fajardo-Lira, H. W. Lee, A. A. Youssefian, V. L. Go and D. Heber, *Nutr Cancer*, 2003, **45**, 226-235.
43. S. Moser, M. Chegeni, O. G. Jones, A. Liceaga and M. G. Ferruzzi, *Food Res Int*, 2014, **66**, 297-305.
44. S. M. Henning, Y. Niu, N. H. Lee, G. D. Thames, R. R. Minutti, H. Wang, V. L. W. Go and D. Heber, *Am J Clin Nutr*, 2004, **80**, 1558-1564.
45. P. Bourassa, R. Côté, S. Hutchandani, G. Samson, & H. A. Tajmir-Riahi, *Photochem. Photobiol. B*, 2013, **128**, 43-49.
46. D. Vitali, I. V. Dragojević and B. Šebečić, *Food Chem*, 2008, **110**, 62-68.

47. V. Kostyuk, A. Potapovich, E. Strigunova, T. Kostyuk and I. Afanas' ev, Arch. Biochem. Biophys, 2004, **428**, 204-208.
48. A. Rawlings, *Exogenous Dermatol*, 2004, **3**, 57-71.
49. Y.H. Wei and H.C. Lee, *Exp Biol Med*, 2002, **227**, 671-682.
50. S. Hsu, W. B. Bollag, J. Lewis, Q. Huang, B. Singh, M. Sharawy, T. Yamamoto and G. Schuster, *J Pharmacol Exp Ther*, 2003, **306**, 29-34.
51. S. T. Boyce, A. P. Supp, V. B. Swope and G. D. Warden, *J Invest Dermatol*, 2002, **118**, 565-572.
52. J. Lee, N. Koo and D. Min, Compr Rev Food Sci F, 2004, **3**, 21-33.
53. S. K. Katiyar, A. Perez and H. Mukhtar, *Clin Cancer Res*, 2000, **6**, 3864-3869.
54. H. Jeon, J. Kim, W. Kim and S. Lee, *Skin Pharmacol Physiol*, 2009, **22**, 137-141.
55. J. Y. Bae, J. S. Choi, Y. J. Choi, S. Y. Shin, S. W. Kang, S. J. Han and Y. H. Kang, *Food Chem Toxicol*, 2008, **46**, 1298-1307.
56. D. M. Palmer and J. Kitchin, *J Drugs Dermatol*, 2010, **9**, 11-15.

Figure legends

Figure 1. Flow chart of present study

Figure 2. Skin surface topography viewed under ultraviolet light (Visioscan). Examination of facial and arm skin of subjects 13. Figure 2A and C represents the facial and arm skin of placebo group, which indicate less skin integrity, whereas figure 2B and D represents the facial and arm skin of 6 months treated with GTPM, which indicate improved skin integrity and texture.



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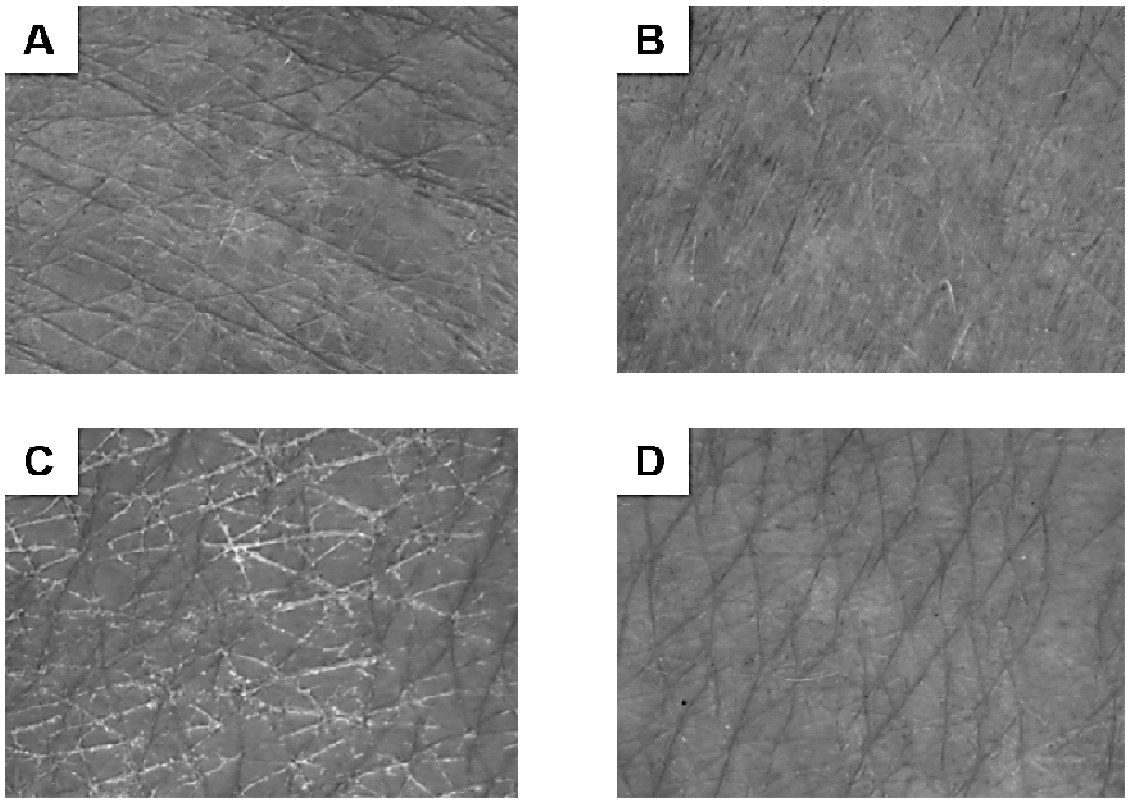

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Table 1. The total phenolics and flavonoids contents in GTPM

	GTPM
Total phenolics[1]	131.37±9.2
Total flavonoids[2]	91.70±0.5

[1]mg gallic acid equivalent / g of GTPM, dry wt.

[2]mg quercetin equivalent / g of GTPM, dry wt.

Table 2. The anthropometric parameters in placebo and GTPM treated healthy subjects

	Group	Weight (Kg)	Body Fat (%)	*BMI(kg/m^2)
Baseline	**Placebo**	60.26 ± 12.47 [a]	25.88 ± 7.89 [a]	22.45 ± 3.82 [a]
	GTPM	59.98 ± 12.57 [a]	25.28 ± 8.31 [a]	22.33 ± 3.85 [a]
3rd Month	**Placebo**	60.03 ± 12.82 [a]	25.26 ± 7.86 [a]	22.34 ± 3.88 [a]
	GTPM	59.95 ± 12.60 [a]	25.19 ± 7.87 [a]	22.35 ± 3.82 [a]
6th Month	**Placebo**	60.03 ± 12.84 [a]	25.23 ± 7.81 [a]	22.34 ± 3.82 [a]
	GTPM	59.93 ± 12.76 [a]	25.04 ± 7.90 [a]	22.23 ± 3.93[a]

Values were expressed as means ± SD (n=44). Data within the same column of each group sharing different superscript letters were significantly different (p<0.05).

*BMI: body mass index

Table 3. Various plasma oxidative indexes in placebo and GTPM treated healthy subjects

	Group	TEAC (µM)	*p*-value*	TBARS (µM)	*p*-value*	Total Phenol (µg/mL)	*p*-value*	GSH (µM)	*p*-value*	Vit-C (µM)	*p*-value*
Baseline	Placebo	0.55 ± 0.01[a]	0.95	1.13 ± 0.36[a]	0.71	3.49 ± 0.20[a]	0.69	18.04 ± 1.30[a]	0.95	1.04 ± 0.47[a]	0.45
	GTPM	0.54 ± 0.01[a]		1.09 ± 0.35[a]		3.51 ± 0.16[a]		18.08 ± 3.51[c]		1.12 ± 0.33[a]	
3rd	Placebo	0.56 ± 0.01[a]	0.02	0.98 ± 0.38[a]	0.022	3.51 ± 0.19[a]	0.21	17.89 ±1.80[a]	0.001	1.00 ± 0.36[a]	0.021
Month	GTPM	0.58 ± 0.01[b]		0.81 ± 0.28[b]		3.56 ± 0.17[b]		21.16 ± 3.22[b]		1.17 ± 0.30[a]	
6th	Placebo	0.56 ± 0.01[a]	0.01	1.07 ± 0.42[a]	0.001	3.50 ± 0.08[a]	0.001	17.63 ± 1.84[a]	0.001	1.07 ± 0.26[a]	0.001
Month	GTPM	0.59 ± 0.01[b]		0.63 ± 0.14[c]		3.75 ± 0.10[c]		23.90 ± 3.28[b]		1.40 ± 0.25[b]	

Values were expressed as means ± SD (n=44). Data within the same column of each group bearing different superscript letters were significantly different (p<0.05). *Student's t-test was used to assess statistical significance between placebo and GTPM

Table 4. Erythrocyte antioxidative enzymes in placebo and GTPM treated healthy subjects

	Group	CAT (IU/g Hb)	*p*-value*	GPx (IU/g Hb)	*p*-value*	GST (IU/g Hb)	*p*-value*	GR (IU/g Hb)	*p*-value*	G-6-PDH (IU/g Hb)	*p*-value*
Base	Placebo	258.91 ± 105.35[a]	0.02	52.90 ± 7.50[a]	0.05	7.16 ± 3.17[a]	0.01	8.90 ± 1.72[a]	0.62	1401.45 ± 286.90[a]	0.244
-line	GTPM	214.49 ± 123.63[b]		49.96 ± 8.66[a]		4.85 ± 1.63[a]		9.16 ± 1.68[a]		1490.94 ± 380.18[b]	
3rd	Placebo	245.18 ± 82.48[a]	0.001	52.21 ±6.70[a]	0.56	6.85 ± 2.58[a]	0.36	8.76 ± 1.49[a]	0.02	1385.60 ± 381.16[a]	0.07
Month	GTPM	324.19 ± 101.13[b]		53.25 ± 9.44[b]		5.18 ± 2.08[a]		9.63 ± 1.98[a]		1557.20 ± 469.78[b]	
6th	Placebo	253.94 ± 79.80[a]	0.001	52.52 ± 9.47[a]	0.21	7.65 ± 2.85[a]	0.09	9.17 ± 1.96[a]	0.001	1364.17 ± 412.86[a]	0.001
Month	GTPM	351.68 ± 100.07[b]		55.04 ± 8.71[b]		8.74 ± 2.97[b]		11.86 ± 2.50[b]		1754.61 ± 399.75[a]	

Values were expressed as means ± SD (n=44). Data within the same column of each group bearing different superscript letters were significantly different (p<0.05). *Student's t-test was used to assess statistical significance between placebo and GTPM

Table 5. Skin examination in placebo and GTPM treated healthy subjects

Parameters	Duration	Placebo		GTPM	
		Face	Arm/T-zone	Face	Arm/T-zone
Skin Elasticity	Baseline	0.860±0.06[a]	0.891±0.05[a]	0.871±0.06[a]	0.893±0.05[a]
	6th Month	0.861±0.08[a]	0.889±0.05[a]	0.869±0.06[a]	0.891±0.04[a]
Skin Moisture	Baseline	46.45±13.92[a]	41.65±6.70[a]	44.70±14.14[a]	40.22±7.43[a]
	6th Month	46.72±14.54[a]	40.83±7.66[a]	44.61±14.78[a]	43.33±6.86[a]
Surface water	Baseline	8.73±2.28[a]	5.33±1.65[a]	8.77±3.17[a]	4.88±1.93[a]
loss (g/m^2/h)	6th Month	8.75±3.28[a]	5.30±2.59[a]	8.75±3.24[a]	4.85±2.01[b]
Skin Wrinkles	Baseline	35.15±3.26[a]	36.95±3.22[a]	35.74±3.81[a]	36.93±3.61[a]
	6th Month	35.50±3.89[a]	36.13±3.58[a]	35.22±3.09[a]	36.21±4.48[a]
Skin Melanin	Baseline	214.01±37.56[a]	196.76±40.91[a]	210.44±36.13[a]	194.58±37.81[a]
Index	6th Month	212.93±37.02[a]	195.05±41.25[a]	212.70±40.37[a]	194.79±44.08[a]
Skin Erythema	Baseline	286.13±74.15[a]	238.20±56.97[a]	286.11±75.07[a]	225.68±51.44[a]
Index	6th Month	289.56±80.55[a]	234.67±51.70[a]	286.86±77.74[b]	220.00±53.81[a]

Values were expressed as means ± SD (n=44). Data within the same column of each group sharing different superscript letters ([a, b]) was significantly different (p<0.05).

Table 6. Skin examination in placebo and GTPM treated subjects over 60 years old

Parameters	Duration	Placebo		GTPM	
		Face	Arm/T-zone	Face	Arm/T-zone
Skin Elasticity	Baseline	0.806±0.04[a]	0.800±0.07[a]	0.842±0.06[a]	0.803±0.08[a]
	6th Month	0.832±0.086[a]	0.803±0.08[a]	0.807±0.06[b]	0.853±0.06[b]
Skin Roughness	Baseline	2.36±1.06[a]	1.96±0.78[a]	2.37±1.07[a]	1.89±0.81[a]
	6th Month	2.37±1.07[a]	1.89±0.81[a]	2.39±0.58[a]	1.58±0.73[a]
Skin Erythema	Baseline	260.21±47.17[a]	231.68±33.06[a]	260.12±80.73[a]	224.53±52.96[a]
Index	6th Month	262.37±42.15[a]	229.40±31.93[a]	264.25±73.01[b]	232.24±53.14[a]
Skin Wrinkles	Baseline	39.63±3.83[a]	42.00±3.67[a]	41.07±4.53[a]	42.08±3.63[a]
	6th Month	41.07±4.53[a]	42.08±3.63[a]	37.20±3.61[b]	37.72±3.99[b]

Values were expressed as means ± SD (n=44). Data within the same column of each group sharing different superscript letters ([a, b]) was significantly different (p<0.05).