



Research Article

Evaluation of safety and efficacy of *Stevia* moisturiser gel by clinical trialKUNTAL DAS^{1*}, RAMAN DANG² AND LALITHA B.R³¹ St. John's Pharmacy College, Vijayanagar, Bangalore-104, INDIA.² Al-Ameen College of Pharmacy, Hosur Main Road, Opp. Lal Bagh Main Gate, Bangalore-27, INDIA.³ Government Ayurvedic Medical College and Hospital, Bangalore-09, INDIA**ARTICLE DETAILS***Article history:*

Received on 09 July 2012

Modified on 20 September 2012

Accepted on 25 September 2012

*Keywords:*Complexion,
Moisturiser gel,
Stevia extract,
Skin softness,
Skin hydration,
Skin pH**ABSTRACT**

The objective of the present comparative study was to evaluate the safety and efficacy of *Stevia* moisturiser gel for the period of single application over 21 days. Sixty subjects were included in this study. They were divided in two groups viz. group -I (control, without *Stevia* extract) and group-II (test, gel containing *Stevia* extract) and each groups contains thirty subjects. They were advised to apply once daily for a period of 21 days. All the subjects were followed up at weekly intervals for a period of 3 weeks. Response to moisturiser gel was evaluated on a scoring system and visual analogue scale. All the subjects were completed the study and significant results observed with respect to reduction in dark complexion, increased in skin softness, skin glow. Measurement of skin hydration after single application of *Stevia* gel during 3 weeks daily was significantly higher (P value <0.001, paired t-test) than the control skin. The gel is completely free from any reaction due to sun light. None of the volunteers experienced neither any hypersensitivity reactions nor in changes of skin pH and compliance to the use of formulation was potent. Finally, concluded that the *Stevia* gel could be suggested as a safe and beneficial moisturizer for human application.

© KESS All rights reserved

INTRODUCTION

Stevia is the nature's sweetest gift belongs to the family Asteraceae. *Stevia* is a native to South America (Paraguay, Brazil)^[1] but extensively grown in places like Central America, Israel, Australia, Japan and China^[2]. It is used for the treatment of various conditions such as cancer^[3], diabetes^[4], obesity, cavities, hypertension^[5], fatigue, depression, dental preparations and in cosmetic^[6]. It possesses hypoglycemic, hypotensive, vasodilating, taste improving, sweetening, anti-fungal, anti viral, anti inflammatory, anti bacterial^[7] properties and increases urination function of the body. It has been found to be non toxic, non addictive, non carcinogenic, non mutagenic, non teratogenic and is devoid of genotoxic effect^[8]. Hence *Stevia* is a new promising renewable raw stuff in the world market. It has captured growing interest as a potential source of natural non-caloric sweeteners for use as a substitute for synthetic sweeteners.

The FDA's approval of *Stevia* after several assurance of quality, safety in food and drink, its application and development capabilities to support successful product innovations that opened the ways for this market's explosion. New product activity has accelerated in recent years, and since most categories with *Stevia* applications remain untapped. The focus of the study is not only to substantiate *Stevia*'s effect as calorie substitute and sweetener but also to justify the multiple uses of stevioside in particular. The *Stevia* extracts are used for various medicinal purposes as a drug and as sugar supplement (due to presence of stevioside, rebaudiosides) in various food products, but its uses in cosmetic field is not still explored. It's time to streamline necessary forces to have access to *Stevia*. Initiative needs to be under taken to promote this natural sweetener and create product awareness. This would be the right approach to unlock the sweetness of this herb in our day to-day life. Its skin toning activity was reported earlier^[9] but not explored largely and hence a successful attempts have been made for the first time to establish the role of *Stevia* as skin tonner.

***Author for Correspondence:**

Email: drkkdsd@gmail.com

MATERIALS AND METHODS

Determination of Potency of Herbal gel containing Stevia extract

The potency of *Stevia* herbal gel was determined by using patch test. The patch test was carried out at Government Ayurvedic Medical College, Bangalore under the guidance of Dr. B.R Lalitha. Patch test was used to identify agents responsible for allergic contact dermatitis^[10].

Materials

Test article: *Stevia* moisturiser gel which contain crude Aqueous *Stevia* extract.

Preparation of Stevia gel:

One gram of Carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring and kept overnight to get a smooth gel. 2 ml of distilled water was taken and the required quantity of methyl paraben was dissolved by heating on water bath. The solution was then cooled to add PEG 400. Then, the required amount of *Stevia* extract was mixed in the above mixture and its volume was increased to 100 ml by adding distilled water. Finally, full mixed ingredients were mixed properly with the carbopol gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjusting the required skin pH (pH: 6.8-7.0) and to obtain required consistency. The same method was followed for the preparation of the control sample without adding *Stevia* extract. 5.0% *Stevia* herbal gel was prepared as described.

Storage condition

Ambient room temperature and humidity.

Method

12 numbers of healthy volunteers were selected (6 males and 6 females) with no underlying skin disease or skin lesion on the test area for primary single patch testing purpose. The test was performed with the objective of potency of *Stevia* moisturizer.

The test was involve the application of *Stevia* gel (0.5 g) to the skin (distal part of the both forearms) by adhesive tape in one forearm and was kept opened in an another forearm. The applied gel was then left for 48 hours. The skin was then examined for 48 hours, for any responses like an allergic contact dermatitis such as skin redness, skin erythema, skin edema etc. After 48 hours the patches were removed from the closed forearm and an initial reading was

recorded one hour later. The final reading was taken at the end of 48 hours. Same method was followed for opened forearm observation. Following observations were recorded according to the International Contact Dermatitis Research Group system, as follows:

Table 1: Skin reaction with score

Skin reaction	Score
<i>Erythema formation</i>	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema (size of 2 mm)	3
Severe erythema to slight escher formation (injuries in depth)	4
<i>Edema formation</i>	
No edema	0
Very slight edema (raising approx 1 mm)	1
Slight edema (raising approx 2 mm)	2
Moderate edema (raising approx 3 mm)	3
Severe edema (raised more than 4 mm)	4

Table 2: Evaluation of primary dermal Index

Evaluation	Index
No irritation	0.00
Irritation barely perceptible	0.04-0.99
Slight irritation	1.00-1.99
Mild Irritation	2.00-2.99
Moderate irritation	3.00-5.99
Severe irritation	6.00-8.00

Determination of efficacy of Stevia herbal gel as moisturiser

An efficacy of *Stevia* moisturizer gel was performed after determination of safety, by using human patch test. About 60 healthy volunteers (30 male and 30 female) were selected for the present study. 3 g of gel was applied in face and in arms for the period of 21 days to all the volunteers. Before and after application of gel the skin pH was determined at interval of 4 and 8 hours. Various skin parameters like Skin hydration, fairness, glow, reaction to sun light, reduction in marks, absence of dark circles, wrinkles, hyperpigmentation and pimples/pits were evaluated each day before and after gel application.

Observations:**Dermal Observation:**

Primary skin irritation was evaluated by an individual blind to operating conditions. The visual scoring system described by Draize et al. (1944) [11]. The Primary Irritation Index was calculated (Table 1 and 2), based on the sum of the scored reactions divided by 48 (two scoring intervals multiplied by two test parameters multiplied by 12 volunteers).

Evaluation of Stevia herbal gel for moisturiser effect:

This study was an open, prospective, placebo controlled clinical trial.

Inclusion criteria: 60 subjects (30 for control and 30 for test), aged between 20- 40 years, who were willing to give informed written consent were selected for the study. The guideline was tabulated in the table below, Table 3.

Table 3: Method followed for clinical trials

Groups	Test Materials	Subjects
I	Control sample (Without <i>Stevia</i> extract)	30 (15 male and 15 female)
II	<i>Stevia</i> gel (5.0% <i>Stevia</i> extract)	30 (15 male and 15 female)

Grouping was carried out by using random number selection method

Exclusion criteria:

Individual with skin infection over face, individuals on treatment with ultraviolet light, widespread active dermatitis or dermatitis on test area, immuno-compromised individuals and those who refused to give informed written content were excluded from the study. Pregnant and lactating women were also excluded from the study.

Procedure:

All the volunteers who were willing to participate in the trial were screened for inclusion in the study. They were advised to clean the face and forearms area thoroughly and then applied the *Stevia* gel daily for the period of three weeks. Follow up and assessment: All the volunteers were followed up at weekly intervals for a period of three weeks, and the symptom score evaluation were done during each follow up visit. Response to moisturizer gel in terms of skin hydration, fairness, skin softness, glow,

reaction to sun light, reduction in marks/specs, absence of dark circles, wrinkles, pimples/pits, sensitivity, irritation/itching and baggy eyes were evaluated on a 5 points visual analogue scale (0= nil, 1= mild, 2= moderate, 3= good and 4= excellent). Scheduled scale selected depends on new developed KRL (Kuntal, Raman and Lalitha) scoring (Table 4).

Table 4: Skin parameters and index

Skin parameters	Index
Excellent	12-15
Good	8-11
Moderate	4-7
Mild	1-3
Nil	0

Skin hydration:

Generally skin hydration can determine by instrumental method using corneometer. But in our study we have develop a scoring system (KRL score) with respect to scratching on skin to find out white lines on skin. Before and after using *Stevia* gel the white lines were observed. Reduction of white lines in number of subjects have recorded and scored. Further confirmed by corneometer estimation.

Skin pH:

Skin pH was determined with using skin pH meter.

Hyperpigmentation of skin:

A new method for assessing skin color is the Taylor Hyperpigmentation Scale. This scale has been validated and may be used to monitor the treatment of hyperpigmentation in both research and clinical settings. The scale consists of 15 laminated plastic cards representing various skin hues. Each card features 10 bands of increasingly darker gradations of the skin hue, representing progressive levels of pigmentation that also may be present. The individual's skin color first is matched as closely as possible to 1 of the 15 skin hue cards, and then the area of hyperpigmentation is matched to one of the 10 bands. Primary and secondary endpoints: The predefined primary efficacy endpoint was a decrease in the symptom score for dark complexion. The predefined secondary safety endpoints were acute and chronic safety, as assessed by the incidence of adverse events of the volunteers.

Table 5a: Effect of the Stevia gel at the end of the study

Effects	No of subjects (30)	Reduction in dark complexion		Increase in skin softness		Skin glowing effect		Reaction to sun light		Skin hydration	
Excellent	Responders	02	24*	02	25*	04	25*	00	00	02	24*
Good		12		14		15		00		19	
Moderate		10		09		06		00		03	
Mild	Non	05	06	05	05	04	05	00	30*	06	06
Nil	Responders	01		00		01		30		00	

*p<0.05

Table 5b: Effect of the Stevia gel at the end of the study

Effects	No of subjects (30)	Reduction in mark/specs		Reduction of dark circle		Wrinkles		Reduction of Pimples	
Excellent	Responders	00	11	00	17*	00	00	00	02
Good		00		03		00		00	
Moderate		11		14		00		02	
Mild	Non	07	19	06	13	04	30	03	28
Nil	Responders	12		07		26		25	

*p<0.05

Table 6: Measurement of skin pH (Average of three readings± SEM)

Sl.No.	Days								
	7			14			21		
	Before Apply at 9 O'clock	After Apply		Before Apply at 9 O'clock	After Apply		Before Apply at 9 O'clock	After Apply	
	1 O'clock	5 O'clock		1 O'clock	5 O'clock		1 O'clock	5 O'clock	
Control	6.98 ± 0.002	6.98 ± 0.004	6.98 ± 0.002	6.98 ± 0.002	6.98 ± 0.002	6.98 ± 0.004	6.98 ± 0.001	6.98 ± 0.003	6.98 ± 0.001
Stevia gel	6.98 ± 0.002	6.98 ± 0.002	6.98 ± 0.012	6.98 ± 0.004	6.98 ± 0.001	6.98 ± 0.001	6.98 ± 0.002	6.98 ± 0.011	6.98 ± 0.001

Non significant, p>0.05, Average of three readings of thirty volunteers.

Table 7: Corneometer measurement of skin hydration (Mean ± SD)

Items	Mean ± SD (n = 30)								
	7 th day			14 th day			21 st day		
	Base line	9 O'clock	4 O'clock	Base line	9 O'clock	4 O'clock	Base line	9 O'clock	4 O'clock
Stevia gel	63.33± 4.761	92.96± 6.304	91.66± 4.895	60.33± 2.272	92.06± 6.915	90.46± 3.907	60.46± 3.296	90.73± 6.702	89.46± 3.500
Control	62.0± 4.804	62.16± 2.281	60.96± 2.401	59.4± 1.311	61.23± 2.775	60.33± 1.405	60.23± 2.103	61.16± 1.818	60.46± 1.704
Mean difference	1.32	30.6*	30.5*	0.92	30.80*	30.11*	0.22	29.52*	28.8*

* Paired t- test significant (p<0.001)

Statistical analysis:

Analysis of variance with repeated measurement or paired t-test was used to evaluate moisturizing efficacy of the *Stevia* gel. A p-value of less than 0.05 was considered statistical significant difference. Statistical analysis was carried out by using InStat graph pad software 3.

RESULTS

Group -I (Control group) showed no hypersensitivity reaction or change of skin coloration. Group-II (test group) showed reduction of dark complexion, significant increase in skin hydration, softness and skin glowing effect over control. Further no reaction to sun light, moderate reduction in marks, absence of dark circles etc were also observed (Table 5a, 5b). None of them was withdrawn from the therapy either for adverse events or for other reasons. Skin pH was measured for all the volunteers at 7 days intervals with skin pH meter. Results show no significant variation of skin pH (pH 6.8-7.00) when compared with the control (Table 6). Further corneometer estimation confirms the skin hydration property of the *Stevia* gel (Table 7).

DISCUSSION

Many procedures for skin safety testing of newly developed chemicals and finished products exist. In 1944, Draize published a method for assessing skin corrosion and acute irritation in rabbits that has served as the basis for classification of skin corrosion and irritation hazard to human^[12, 13]. However, the Draize test has come under increasing criticism for two main reasons, animal welfare concerns, and the poor predictive validity for human response^[14, 15]. This has led to the emergence of the development of new ethical testing and risk assessment strategies in human subjects but cosmetic products and ingredients do not usually cause acute irritation to the consumer. Water based *Stevia* concentrate having ability to heal numerous skin problems, including acne, seborrhea and dermatitis. It is also seen that placing *Stevia* in cuts and wounds bring more rapid healing without the scarring⁹. Looking at that the present study also revealed the same activity when *Stevia* gel was prepared for skin moisturizer. The present study revealed the potency of *Stevia* gel after applied for the period of 48 hours with occlusive patch test and open occlusive testing and showed no positive adverse reactions. This may be because of *Stevia* leaves contain below detectable limits of heavy metals and other toxic materials. Efficacy

of gel was also determined. Interpretation of results revealed that there are significant increases in skin hydration after single application of *Stevia* herbal moisturizer, which are further increased and maintained after regular application of gel upto 3 weeks. It showed no adverse reaction in sun light but it minimize the skin tan, dark circle and improve skin fairness, softness and glow to the skin by acting as coolant. Results of our study are in agreement with the report by Li *et al.* (2001)^[16] and even due to the properties of the active constituents present in the *Stevia* leaves. The test material contains potent active constituents like stevioside, rebaudioside, dulcosides, sesquiterpene etc. It was proposed that the gel containing products improved skin hydration possibly by means of a humectants mechanism^[17]. Generally moisturiser gels have no effect on minimizing of wrinkles and pimples but interestingly the *Stevia* gel also showed very mild reduction in wrinkles and pimples with application for the period of 21 days (Table 5b). This may be due to antioxidant^[18, 19] and antimicrobial^[7, 20] properties reported by the *Stevia* leaves. However, long term application of the gel is recommend helping in reduction of wrinkles and pimples.

CONCLUSION

Differences in plant composition due to geographic location as well as differences in gel extraction methods and sample preparation techniques have contributed to discrepancies in the results obtained from many studies in terms of the chemical composition and biological activities of *Stevia* leaf gel. Although the findings of the present research has demonstrate that the bases that were used for Group-I as control sample, were inactive against any skin adverse reaction but moisturizer *Stevia* gel show effective in lightening the skin complexion, and improved skin hydration, skin soft and smooth. The gel also showed completely free from any reaction due to sun light. None of the volunteers experienced neither any hypersensitivity reactions nor in changes of skin pH (Group-II). Therefore, it may be concluded that the test medication, moisturizer *Stevia* gel is safe and efficacious for human use. Not only that, this study can be helpful for upcoming researchers to select this herb for the formulation and evaluation of other cosmetic applications which can be claimed for their efficacy with scientific data, which shall further give strength to our herbal and cosmetic industries eminence in global market.

REFERENCES

- [1] Bertoni MS. Le Kaa He-e. Sa nature et ses propriétés. *Ancient Paraguayos*. 1905; 1(5) : 1-14.
- [2] Sharma N, Kaushal N, Chawla A, Mohan M. *Stevia rebaudiana*—A review. *Agribios*. 2006; 5: 46-48.
- [3] Yasukawa K, Kitanaka S, Seo S. Inhibitory effect of stevioside on tumor promotion by 12-O-TCA in two stage carcinogenesis in mouse skin. *Biol. Pharma. Bull.* 2002; 25: 1488-99.
- [4] Lailerd N, Saengsirisuwan V, Slonigar JA. Effects of stevioside on glucose transport activity in insulin-sensitive and insulin-resistant rat skeletal muscle. *Metabolism*. 2004; 53: 101-07.
- [5] Dyrskog SE, Jeppensen PB, Colombo M, Abudula R. Preventive effects of a soy-based diet supplemented with stevioside on the development of the metabolic syndrome and type 2 diabetes in Zucker diabetic fatty rats. *Metabolism*. 2005; 54: 1181-88.
- [6] Mowrey D. Life with Stevia: How sweet it is! Nutritional and medicinal uses. *Op Cit*. 1992a; 1-14.
- [7] Ghosh S, Subudhi E, Nayak S. Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. *Int J Integr biology*. 2008; 2: 27-31.
- [8] Klinsukon T, Pimbua J, Panichkul T. Stevioside, a natural sweetener from *Stevia rebaudiana* Bertoni: Toxicological evaluation. *Thai J Toxicology*. 1988; 4: 1-22.
- [9] Mowrey D. Life with Stevia: How sweet it is. *Op. Cit*. 1992b; 12.
- [10] Jirova D, Liebsch M, Basketter D, Spiller E, Kejlova K, Bendova H, Marriott M, Kandarova H. Patch test against allergic contact dermatitis. *Jap. Soc. Alt. Ani. Exp.* 2008; AATEX 14 (Special Issue), 359-65.
- [11] Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharm. Exp. Therap.* 1944; 82: 377-90.
- [12] Nixon GA, Tyson CA, Wertz WC. Interspecies comparisons of skin irritancy. *Toxicol. Appl. Pharmacol.* 1975; 31: 481-90.
- [13] Patil SM, Patrick E, Maibach HI: In: Marzulli FN, Maibach HI, editors. *Dermatotoxicology*. 5th ed. Washington DC: Taylor-Francis, 1996; 411-436.
- [14] Campbell RL, Bruce RD. Comparative dermatotoxicology: I. Direct comparison of rabbit and human primary skin irritation responses to isopropylmyristate. *Toxicol Appl. Pharmacol.* 1981; 59: 555-63.
- [15] Robinson MK, Perkins MA. A strategy for skin irritation testing. *Am. J. Contact Dermat.* 2002; 13: 21-29.
- [16] Li F, Conroy E, Visscher M, Wickett RR. The ability of electrical measurements to predict skin moisturization II. Correlation between one-hour measurements and long-term results. *J Cosmet Sci.* 2001; 52: 23-33.
- [17] Dal'Beló SE, Gaspar LR, Berardo G, Maia Campos PM. Moisturising effect of cosmetic formulations containing *Aloe vera* extract in different concentrations assessed by skin bioengineering techniques. *Skin Res. Technol.* 2006; 12: 241-46.
- [18] Tadhani MB, Patel VH, Subhash RJ. *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. *Food Comp. Anal.* 2007; 20: 323-29.
- [19] Shukla S, Mehta A, Bajpai VK, Shukla S. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. *Food. Chem. Toxicol.* 2009; 47: 2338-43.
- [20] Das K, Dang R, Gupta N. Comparative antimicrobial potential of different extract of leaves of *Stevia rebaudiana* Bert. *Int. J. Nat. Eng. Sci.* 2009; 03(01): 59-62.