

Formulation and Evaluation of A Novel Herbal Gel Of Stevia Extract

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Abstract

Background: The aim of this study was to formulate and evaluate the noble herbal moisturise gel containing Stevia extract.

Methods: The cosmetic gel formulation was designed by using aqueous extract of Stevia rebaudiana leaves in varied concentrations (2.5% and 5.0%) and evaluated using physiological measurements in comparision with a control placebo gel. The initial physicochemical parameters of formulations SF-I and SF-II, i.e. pH, viscosity, spreadability, extrudability and stability were examined. Furthermore, both formulations were studied for toxicity and skin irritancy on animal model.

Results: This study revealed that the formulation containing 2.5% Stevia extract showed comparatively better stability than other formulations and the control sample. Results showed that there were no toxicity and no skin irritation according to evaluated various parameters when compared to the control formulation.

Conclusion: This formulation of Stevia extract could be suggested as a safe and beneficial moisturiser. (*Iran J Dermatol 2009;12: 117-122*)

Keywords: aqueous extract; evaluation; gel formulation; Stevia rebaudiana; toxicity study

Introduction

A gel is a semisolid system of at least two interpenetrating phases: a gelling agent and a liquid. Gels that contain water are called hydrogels, while those that contain an organic liquid are called organogels. Hydrogels, in the broad sense, include the matrix of water - soluble materials such as cellulose derivatives and natural gums. Gels are used pharmaceutically as lubricants and as carriers for spermicidal agents¹ and other drugs for their local effects and percutaneous absorption².

Herbals are the oldest form of primary healthcare which were used by all cultures throughout history. They were an integral part of the development of modern civilization. Of late, Stevia is a plant indigenous to South America (Paraguay and Brazil) and belongs to the family of Compositae³. The Asian markets consume over 85% of the global supply of the fluffy white crystalline Stevia extracts. The current extract market is 1.5 million kg, processed from 12 million kg of Stevia leaf and is used for various preparations. Stevioside is one of the important

active constituents which is abundantly available in the leaf of Stevia (5-10% of dry weight basis) and is 300- 350 times sweeter than sucrose⁴. In the medicinal field, it has hypoglycemic, oral contraceptive, cardiovascular, and antimicrobial activities. It is also used for weight loss, digestive and skin problems^{5,6,7}. There are scanty reports on Stevia in the form of a herbal gel preparation. In one study, gel formulations containing 5, 10, 15% crude Stevia extract and 5 and 10% of pure stevioside were prepared and their physical stability were established⁸. Looking at that, as part of a Ph.D. study, successful attempts were made for the first time in India to establish the role of Stevia as a skin tonner. Hence, various formulations were prepared from the cultivated Stevia in South India (Ripponpet, Shimoga Dist, Karnataka) under an acidic soil, pH 6.10, and tested for pH, stability, viscosity, tonicity, spreadibility and other parameters. Further preclinical study was carried out to investigate toxicity and irritancy (with only single Stevia herb) that would cover all the aspects of improving healthy skin.

Materials and Methods

Materials

Cuttings of *Stevia* plants, collected from Ankur Nursery, Ripponpet (Shimoga, Karnataka), India, were used as a test plant for the present study. As part of a Ph.D. research study, a field experiment was conducted from November 2005 to May 2006 at the Ripponpet, Shimoga on acidic soil reaction (pH 6.10). After six months of field experiment, the plant samples (leaves) were collected and oven dried at 60°C for 6 hours. The dried leaves were stored at 4°C and were used for further preparation of the herbal extract.

Preparation of Stevia extract

50 g of dried *Stevia* leaves was extracted with distilled water using reflux condenser for 6 hours after standardization of the method. Oven temperature was maintained at 45°C. Extract was collected and filtered using Whatman No 1 filter paper and the filtrate was then subjected to evaporation under reduced pressure to get a soft extract and stored in labeled sterile screw capped bottles at -15°C. The yield of the aqueous extract of the leaf was found to be 32 g% and this extract was used for preparing the herbal gel.

Different combinations of the *Stevia* crude extract (2.5% and 5.0%) were tried with different types of polymers (Carbopol 934, carbopol 940, HPMC, Sodium CMC) using various formulae. The following few combinations with carbopol 934 resulted in the best gel formulation which was non greasy, smooth and stable. A control sample was also prepared for animal study to check the activity of control ingredients.

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 dropwise 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. 2.5% and 5.0% *Stevia* herbal gel were prepared as described. Physical parameters such as color, appearance and feeling on

application were recorded. PH of the gel was measured and recorded using a PH meter (Elco Pvt Ltd, India).

Extrudability

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair) ⁹.

Spreadability

The formulation was placed over the glass plate of 20cm X 5cm. Another glass plate of the same dimension was placed on the top of the gel such that the formulation was sandwiched between the two slides by placing a weight of 100 gr uniformly on the slides. The weight was removed and the excess of gel was scrapped off. Two slides in position were fixed to a stand at a 45° angle without the slightest disturbance so that only the lower slide was held firmly by the clamp, allowing the upper slide to slip off freely with the help of 20 gr weight tied to the upper slide. The time taken for the upper slide to separate away from the lower glass plate under the direction of the weight was noted as per ICH guidelines ¹⁰. Experiment was done in triplicate and spreadability was calculated as follows:

$S = M \times L / T$, Where, S = Spreadability, L = Length of the glass plate.

W=Weight tied to the upper plate, T = Time taken (sec).

Viscosity

Viscosity of the gel was measured at 25°C ± 2°C, using brookefield viscometer (Model-RVTP) with spindle type-7.

Stability study

The International Conference on Harmonization (ICH) harmonized tripartite guidelines on stability testing of new drug substances and products was issued on October 27, 1993 and we used this guideline to assess the gel stability in our study ^{10,11}.

Primary Dermal Irritation Index (PDII)

Dermal irritation is the production of reversible damage to the skin following the application of a test substance for up to 4 hours. Primary dermal irritation index (PDII) is a method for classifying

topical formulations into various categories based on acute toxic reactions observed upon single application of a formulation on skin. Based on the PDII score, the formulation can be graded as irritating or non-irritating.

Selection and maintenance of animals

Healthy young male albino rabbits, weighing 1.5 – 2 kg at the start of the experiment, were used as the experimental animals in the present study (Clinical Ethical clearance No: AACP/IAEC/P-38/2006). The animals were housed together in a clean tank which was spacious enough for the free movement of the animals and accommodation to hold drinking water and feed. Room temperature was $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$, humidity was 45-55% with a light period of 12 h (06.00 to 18.00). The animals were fed with commercially available standard pellet chow (Amrut Feeds, Bangalore) and filtered tap water.

Preparation of animals

Approximately 24 hours before the test, fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin and only animals with healthy, intact skin were used for the study.

Application of the herbal gel

Half a gram of the herbal gel, as the test substance, was applied to an area of approximately 6 cm² of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a suitable semi-occlusive dressing for 4 hours and was then removed. At the end of the exposure period, i.e. 4 hours, residual test substance was removed without altering the existing response or the integrity of the epidermis. Observations were recorded an hour after the removal of the patch. Control animals were prepared in the same manner and 0.5 gram of the gel base, i.e. gel formulated using all the ingredients except the herbal mixture, was applied to the control animals and observations were made similar to the test animals.

Both the control and the test animals were observed every day for any occurrence of skin irritation or toxic reactions such as edema or erythema. Per observation of skin, a value between 0 and 4 was recorded where 0 meant no skin erythema and eschar formation and 1, 2, 3 and 4 stood for very slight, well defined, moderate and severe erythema to eschar formation, respectively. It also scored from 0–4, where 0 stood for no edema and 4 stood for severe edema.

Primary Dermal Irritation Index (PDII) =

$$\frac{\text{PDII observed on 12+24+48+72 hrs}}{4}$$

Classification system based on PDII

< 0.5: non-irritating, 0.5-2.0: slightly irritating, 2.1-5.0: moderately irritating and >5.0: severely irritating.

28 days repeated dose dermal toxicity of the developed herbal gel formulation:

28 days repeated dose dermal toxicity of the herbal gel formulations (2.5% and 5.0%) was carried out to study the toxicity of the herbal formulation upon repeated application on the rabbit skin. The study was conducted to evaluate the cumulative toxicity of the herbal gel upon repeated application not only on the skin but also on behavioral, hematological and biochemical parameters. Histopathology of vital organs such as kidney, liver and heart was also studied.

Both the control and the test animals were observed every day for any occurrence of skin toxic or irritation reactions such as edema or erythema.

Body weight analysis

Initial weights of both control and test animals were recorded on the day of commencement of the study and the final weights of all the control and test animals were recorded on the 28th day before withdrawal of the blood. Changes in the weight of the test animals were compared with that of the control animals.

Hematological analysis

Blood samples were collected from by vein puncture of all the test and control rabbits on the 14th and on the 28th day of the study. Estimation of haemoglobin percentage was done using haemocytometer.

Biochemical analysis

For determining Blood sugar, Total cholesterol, creatinine, urea, total and direct bilirubin, Protein, SGOT, SGPT, Alkaline phosphatase and acid phosphatase, blood samples were collected separately from each control and experimental animal by retro orbital puncture on the 14th and 28th day of the study.

Organ weight analysis

After 28 days, both the test as well as the control animals were humanely sacrificed after collecting blood for hematological and biochemical analysis. Vital organs like liver, kidney and heart of each animal were isolated. The isolated organs were

observed for their morphology such the presence of any kind of lesion and every individual organ of each animal was weighed. The results were compared with the control animals.

Histopathology of heart, liver, skin and kidney

Heart, liver and kidney tissues isolated from individual animals were fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Paraffin sections (3 µm) were cut on glass slides and stained with hematoxylin, eosin, periodic acid Schiff reagent and examined under a light microscope for pathological changes.

Results

The herbal gel was prepared and subjected to evaluation of the various parameters. The herbal gel was dark greenish in color and translucent in appearance and had a cool and smooth feeling on application. PH also maintained constant throughout the study which was found to be 6.96 to 6.98 and the gel was non-irritant upon application on the skin. Extrudability and spreadability were also measured and found to be less variant than the initially prepared gel after performing stability study (table1,2). The initial viscosity of developed gels were measured using the Brookefield viscometer at different rpm and respective viscosities were recorded at 25°C using Spindle#7 (table 3). Furthermore, an stability test was carried out for three months and results revealed that the gel that contained 2.5 % *Stevia* extract showed a better stability than 5.0 % *Stevia* extract. Initial viscosity for gel containing 2.5% *Stevia* extract was

24580cps at 50 rpm and after the stability study, there was not much variation after testing at different temperature conditions but variations were observed in the gel contained 5.0 % *Stevia* extract. The reason was that 5.0% *Stevia* extract contained more water which reduced the viscosity.

The total scores for skin irritation in terms of erythema and edema was calculated after 12, 24, 48 and 72 hours according to OECD scoring system. Results revealed the developed herbal formulation did not cause any erythema or edema on the intact rabbit skin when observed for 72 hours. The Primary Dermal Irritation Index (PDII) of the formulation was 0.00; hence, according to OECD guidelines the formulation can be classified as non-irritant to the rabbit skin. No clinical signs of dermal toxicity were observed in any of the animals treated with the test substance upon repeated application of the herbal gel for up to 28 days.

The control and the experimental rabbits showed no signs of tremor, convulsions and reflex abnormalities. No muscular numbness of the hind and forelegs, salivation or diarrhea was observed. The food intake per day was also normal during the 28 days repeated dose dermal toxicity evaluation.

Body weight of all the control and treated rabbits increased and changes of body weight were statistically calculated. There were four groups including the control group. Hence, simple one way ANOVA was used for statistical analysis and the results are shown in table 4.

Hematological profiles of the experimental rabbits were studied after the repeated

Table 1. Extrudability for all the formulations at the time of gel Preparation (Mean ± SEM)
Mean of three tubes (Initial month)

Formulations	Extrudability			Grade
	Net wt of formulation in tube (g)	Wt of gel Extruded (g)	Extrudability amount in percentage	
Control	12.58 ± 0.008	9.23 ± 0.011	73.37 ± 0.010	++
SF-I (2.5 %)	12.34 ± 0.011	11.32 ± 0.014	91.73 ± 0.005	++++
SF-II (5.0 %)	12.37 ± 0.011	11.27 ± 0.008	91.10 ± 0.014	++++

Foot Note: Excellent = + + + + ; Good = + + + ; Fair = + + , SEM = Standard Error Mean

Table 2. Spreadability for all the formulations at the time of gel Preparation
Mean of Three readings (Mean ± SEM) (Initial month)

Formulation	Time in Second			Mean Time in Sec	Spreadability
	1	2	3		
Control	5	6	6	5.66 ± 0.011	17.67± 0.012
SF-I (2.5 %)	3	4	3	3.33± 0.015	30.03 ± 0.012
SF-II (5.0 %)	4	4	5	4.33± 0.012	23.09± 0.015

SEM = Standard Error Mean

Table 3. Viscosity (Spindle# 7) for all the formulations at the time of gel Preparation (Initial month)

Formulation	RPM	Torque (%)	Viscosity (cps)
Control	50	36.7	29410
	75	41.1	21930
	100	44.5	17800
	150	50.0	13360
SF-I (2.5 %)	50	30.8	24580
	75	33.5	18770
	100	36.7	15760
	150	39.3	12507
SF-II (5.0 %)	50	27.6	22080
	75	30.0	16053
	100	32.1	13460
	150	35.1	11787

application of herbal gel daily for 28 days. Hematological parameters such as total counts of RBC and WBC, differential count of WBC and hemoglobin percentage were normal before treatment and after 14 and 28 days of application of the herbal gel. No detectable changes were observed in the values of these parameters compared to the control groups.

Biochemical parameters of blood such as blood sugar, total cholesterol, creatinine, urea, total and direct bilirubin, total protein, SGOT, SGPT, alkaline phosphatase and acid phosphatase of both test and the control rabbits were evaluated for any change in these parameters due to the application of the herbal gel with respect to the control rabbits. The changes were statistically insignificant. These results indicated that the herbal gel had no adverse effects on the biochemical parameters of the blood.

Organ weights of the control and the test animals did not show much variation.

Histopathological study of the animals' heart, kidney, liver and skin was carried out and compared with the control group which showed no abnormalities or any deformation of the organs, indicating the safe and non toxic nature of the

herbal gel containing Stevia extract.

Discussion

There is a growing demand for herbal cosmetics in the world market and they are invaluable gifts of nature. Therefore, we tried to make an herbal gel containing Stevia extract at two different concentrations (2.5% and 5.0%) and tested them for all the physicochemical parameters of gel. Our study indicated that the developed herbal formulation consisting 2.5% Stevia extract was comparatively better than other one after the stability study; however, both formulations were non irritant and did not show any toxicity when applied daily for 28 days in animals. Further, histopathology reports confirmed the safety of Stevia gel as a skin moisturizer. Stevia (*Stevia rebaudiana* Bertoni) seems to show several biologic roles such as non-caloric sweetener, anti-obesity or antioxidant effect especially in animal studies¹²⁻¹⁴. Moreover, there are some reports on antimicrobial effect of Stevia extract or its hypoglycaemic effects^{15,16}. However, To best of our knowledge, there was no structured study of topical Stevia extract gel development in the literature and this assay seems one of the first one that assess the potential of Stevia extract as a competent and safe topical emollient. However, further structured study, especially in human phase, would be beneficial to assess its usefulness more exactly.

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Table 4. Effect of repeated topical application of herbal gel for 28 days on the body weight of rabbits.

Group	Body weight(kg) before treatment $M_1 \pm SD_1$ (N=3)	SEM	Body weight(kg) 28 days after treatment $M_2 \pm SD_2$ (N=3)	SEM	P Value
Control	1.67 \pm 0.010	0.005	2.15 \pm 0.015	0.008	NS, P> 0.05
Treated (2.5%)	1.64 \pm 0.015	0.008	2.16 \pm 0.005	0.003	NS, P> 0.05
Treated (5.0 %)	1.66 \pm 0.010	0.005	2.15 \pm 0.015	0.008	NS, P> 0.05

M_1 and M_2 = Mean values of the samples, SD_1 and SD_2 = Standard deviations of control and experimental group respectively, N= number of rats. NS = Not significant (P> 0.05).

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