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Research Article

Evaluation for safety assessment of formulated vanishing cream containing aqueous *Stevia* extract for topical application

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ABSTRACT

In the present work, herbal vanishing cream based drug formulations (2.5% and 5.0%) were designed and prepared for the skin moisturizer with *Stevia* white extract. The creams were O/W emulsion based formulations containing suitable combination of oil phase and aqueous phase along with preservatives. Both the creams were non greasy, coolant and pearl like appearance. They were subjected to various physicochemical parameters i.e. pH, viscosity, spreadability, tube extrudability and drug content studies. Stability studies of creams were also done at different temperature for the period of 3 months as per ICH guideline and results revealed both the formulations showed good stability over control preparation. Amount of active constituent present, pH, spreadability, tube extrudability of the formulation were found to be 7.47 mg and 3.64 mg; 6.70-6.80, 16.11- 16.12 gm.cm/sec; 92.38% - 92.40 % for 5% and 2.5% *Stevia* cream respectively. Furthermore, both the formulations were studied for primary skin irritation test in rabbit and 30 numbers of healthy human volunteers for 48 hours and observed for skin rashes, inflammation, itching or redness on applied portions. Results revealed no adverse skin reactions with all the formulations. It was proved that vanishing cream containing *Stevia* extract is pleasant, effective, easily washable and completely safe for human use.

INTRODUCTION

Dry skin is a very common skin condition characterized by abnormal or excessive dryness of skin. Dry skin has a low level of sebum and can be prone to sensitivity. The skin has a parched look caused by its inability to retain moisture. The condition is also known as xeroderma. Chapping and cracking are signs of extremely dry, dehydrated skin. Symptoms of dry skin include discomfort from skin tightness and itching. In addition, external factors are the most common underlying cause and they include cold temperatures and low humidity, especially during the winter when central heaters are used.

Internal factors include overall health, age, genetics, family history and a personal history of other medical conditions like asthma, allergies, and atopic dermatitis.

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Dry skin may also be a side effect of some medications or a symptom from an underlying physiological disorder, like an overactive or underactive thyroid gland or Sjogren's syndrome[1, 2] . Many of the medicines are available in the market to treat the dry skin but they are much more expensive. Patient compliance is often poor as currently available medications are often greasy, sticky, strong odorous, difficult to apply and require frequent application. In proposed work it was planned to prepare vanishing cream formulation with *Stevia* white extract to treat dry skin.

Stevia rebaudiana is a plant indigenous to South America (Paraguay and Brazil) and belongs to the family Compositae [3]. 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 sucrose[4]. 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]. *Stevia* herbal gel was prepared and evaluated for skin tonner[8,9] but scanty reports on *Stevia* in the form of a herbal vanishing cream preparation was forced to direct toward skin moisturizer application. Hence the attempt was made first time in India to establish the role of *Stevia* as a skin moisturizer by prepared vanishing cream. The formulation was nongreasy, water removable and increases patient's compliance. The formulation was called vanishing creams because it disappear when rubbed on the skin. The base of the preparations was mainly stearic acid. A part of stearic acid was saponified with an alkali and rest was emulsified with a soap in a large quantity of water. The high quality stearic acid provides an oil phase, which melts above body temperature and crystallizes in suitable form, provides an invisible and non-greasy film and can produce a very attractive appearance[10, 11].

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. 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. Chemicals like Stearic acid, Methyl paraben and Propyl Paraben – Loba Chemie Pvt. Ltd., Mumbai., Glycerin and Potassium Hydroxide- Qualigens Fine Chemicals, Mumbai and other chemicals used were of analytical grade.

Preparation of *Stevia* extract

250 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. The recovered extract was passed through a macroporous ion exchange resin on which *Stevia* is absorbed. The *Stevia* is then eluted with ethanol. Further *Stevia* solution was decolorized by passing through a mixed bed containing both cation and anion ion exchangers. The decolorized *Stevia* solution is concentrated in an evaporator under reduced pressure to get the white powder of *Stevia* extract (80 g %).

Preparation of vanishing cream formulation

Vanishing creams are o/w emulsion based preparations containing aqueous phase and oil phase. Ingredient of oil phase (A) was melted in a beaker by using water bath on constant stirring. Components of aqueous phase (B) were mixed together and warmed to about same temperature of oil phase (up to 70^0 C). The preservative propyl paraben and methyl paraben was added in to aqueous phase after dissolved in little quantity of warm water. Then oil phase was added to water phase little by little on constant stirring and purfume was added to it when the temperature was 35^0C - 40^0 C. During congealed the mixture appeared as pearl like semi solid mass. Further required quantity of the preparation was taken out from total formulation and specified amount of white powdered *Stevia* extract was mixed uniformly by levigation method to get 2.5% and 5.0% *Stevia* vanishing cream (Table 1).

Table 1: Ingredients for the vanishing cream formulation

Sl.No		Ingredients	Quantity (g)
A	1.	Stearic acid	24
B	2.	Glycerin	10.5
	3.	Potassium hydroxide	1.35
	4.	Methyl Paraben	0.10
	5.	Propyl Paraben	0.05
	6.	Purified water	64
	7.	Purfume	q.s.

Evaluation of cream

The creams were evaluated for pH, content of active component in creams by HPLC method, viscosity, spreadability, tube extrudability and stability studies. Primary skin irritation tests were conducted on experimental animals and healthy human volunteers to evaluate the safety and efficacy of creams.

Determination of pH

Accurately weighed 5 g. of the cream was dispersed in 45 ml. of water to determined the pH of the suspension at 27°C using digital pH meter[12].

Determination of active constituent (Stevioside) in creams by HPLC method

The method was followed as per the method described by Bovanova et al., 1998[13]. C-18 Column was used. Methanol: water (80:20) mobile phase was used with the flow rate of 1.5 ml/min (injection: 10µl) and the peaks were detected at 210 nm at the room temp of 25^{0}C. Further the content of stevioside in creams was estimated by compared with standard.

Viscosity

The viscosity of formulated vanishing creams was measured by Brook field Viscometer (Model-RVTP) with spindle type-7 at room temperature of 25^0 C.

Spreadability

The therapeutic efficiency of the formulation depends on its spreading value. Hence, determination of spreadability is very important in evaluating topical application characteristics in terms of the extent of area to which the topical application spreads on application to skin on the affected parts. 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 45º 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[14]. Experiment was done in triplicate and spreadibility was calculated as follows:

$$S = \frac{M \times L}{T}$$

Where, S = Spreadibility, L = Length of the glass plate. W=Weight tied to the upper plate, T = Time taken (sec).

Tube extrudability

In the present study, the method adopted for evaluating cream formulation for extrudability was based upon the quantity in percentage cream extruded from tube on application of finger pressure. More quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminium collapsible 5 tube with a nasal tip of 5 mm opening and applied the pressure on the tube by the help of finger. Tube extrudability was then determined by measuring the amount of cream extruded through the tip when a pressure was applied on a tube[15]. The percent of the extruded cream was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair)[14].

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 cream stability in our study[14, 16].

Primary skin irritation test

1) Laboratory experimental animals

The animals selected were rabbits (weighing 1.5–2kg, Clinical Ethical clearance No: AACP/IAEC/P- 38/2006). These animals were kept in different cages and supplied with fresh food and water during the test period, 24 hours prior to test, the hair from the neck and thigh region was shaved to expose sufficiently large test area. The test site was cleaned with surgical spirit briefly. Then vanishing cream was applied to the test area. The test site was observed for erythema and edema for 24 h; 48 h; and 72 h after application. This test was conducted to evaluate the irritation caused by the prepared cream on the intact skin of animals.

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.

Application of vanishing creams

Half a gram of the cream, as the test substance, was applied to a an area of approximately 6 cm^2 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 cream base, (without *Stevia* extract) 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 erythma to eschar formation, respectively. It also scored from 0–4, where 0 stood for no edema and 4 stood for severe edema (Table 2 and 3).

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

Where, PDII = Primary Dermal Irritation Index

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 cream formulation

28 days repeated dose dermal toxicity of the vanishing cream formulations (2.5% and 5.0%) were 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 cream 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 commence 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.

2) Healthy Human Volunteers Studies

The prepared formulation showed high compliance in animal studies, thereby prompted to carry out skin irritation studies on healthy human volunteers to check the potency of the cream formulations. The potency of *Stevia* vanishing cream 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[17].

Method

30 numbers of healthy volunteers were selected with the age group between 25-40 years (15 males and 15 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* vanishing cream (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 cream 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 2:

Skin reaction	Score
Erythema formation	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to serve erythea (size of 2 mm)	3
Serve erythema to slight escher formation (injuries in depth)	4
Edema formation	
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 3: 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

RESULTS

The herbal *Stevia* vanishing cream was prepared and subjected to evaluation of the said various parameters. The results of the present research work showed the creams were white in color and pearl like appearance and gave cool and smooth feel on application which was maintained after tested the stability study. P^H of the formulation were shown neutral (6.70-6.80) and the gel was non-irritant upon application on to the skin. An extrudability and spreadibility were also measured and found to be less variation with the initially prepared creams after performed the stability study at 30^0 C (Table 4 and 5). The initial viscosity of formulated creams was measured using Brookefield viscometer at different rpm and respective viscosities were recorded at 25^0C using Spindle#7. Further stability test for three months had carried out and results revealed both the creams contained 2.5 % and 5.0% *Stevia* extract showed constant stability as initial preparation. Initial viscosity for both the creams were compared after stability study of three months and showed not much variation at 30^0 C (Table 6). Further 3 months stability study was carried out at normal room temperature (25^0C) but results were unchanged. Finally the amount content of Stevioside in total cream was estimated with HPLC and revealed 2.5% and 5.0% *Stevia* creams contained 3.64 mg and 7.47 mg of stevioside respectively.

The scores for skin irritation in terms of erythema and edema had totaled for skin for all rabbits at 12, 24, 48 and 72 hours according OECD scoring system. Results revealed the developed herbal creams have not caused 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 classified as a nonirritant to the rabbit skin.

Behavioral Analysis

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

Body and organ weight analysis

The body weights of all the control and treated rabbits were found to be increased and changes of body weights and organ weight were statistically calculated. There are four groups including control group. Hence, simple One way ANOVA has followed for statistical analysis, which are shown non significant.

Table 4: Preliminary studies data of *Stevia* vanishing cream

Formulations	Physical Parameters			pH	Sp. (g. cm/sec)	Ext. (%)
	Color	Appearance	Feel on application			
Control	White	Pearl like	Cool and smooth	6.70	15.14	84.20
Formulation-I (2.5% *Stevia* extract)	White	Pearl like	Cool and smooth	6.79	16.12	92.40
Formulation-II (5.0 % *Stevia* extract)	White	Pearl like	Cool and smooth	6.80	16.14	92.42

Sp. = Spreadability; Ext = Extrudability

Table 5: Stability studies data (at 30^0 C) of *Stevia* vanishing cream (after three months)

Formulations	Physical Parameters			pH	Sp. (g. cm/sec)	Ext. (%)
	Color	Appearance	Feel on application			
Control	White	Pearl like	Cool and smooth	6.68	15.10	84.20
Formulation-I (2.5% *Stevia* extract)	White	Pearl like	Cool and smooth	6.75	16.11	92.38
Formulation-II (5.0 % *Stevia* extract)	White	Pearl like	Cool and smooth	6.78	16.12	92.40

Sp. = Spreadability; Ext = Extrudability

Table 6: Viscosity studies data of *Stevia* vanishing cream

Formulation	RPM	Torque (%)	Viscosity (cps) (Initial)	Torque (%) (After 3 months of Stability study)	Viscosity (cps) (After 3 months of Stability study)
Control	50	32.7	23230	32.3	23180
	75	39.1	19930	37.4	19910
	100	40.4	15200	38.9	15188
	150	46.0	12160	44.6	12143
Formulation-I	50	28.2	20140	27.8	20128
(2.5 %)	75	30.1	15480	30.0	15478
	100	32.4	13460	31.8	13456
	150	37.1	10212	37.0	10210
Formulation-II	50	24.4	22080	24.2	22075
(5.0 %)	75	26.2	14143	26.0	14138
	100	28.8	11460	28.2	11454
	150	30.6	9582	30.1	9579

Table 7: Effect of repeated application of herbal gel for 28 days on hematological parameters of rabbits

Haematological Parameters		C_1 , $M_1 \pm SD_1$, (N=3)		T_1 , $M_2 \pm SD_2$, (N=3)		T_2, $M_3 \pm SD_3$, (N=3)	
		14th Day	28th Day	14th Day	28th Day	14th Day	28th Day
Total RBC (Million/cc)		4.81 ±0.010	4.81 ± 0.023	4.81 ± 0.012	4.82 ± 0.021	4.84 ±0.020	4.84 ± 0.012
Total WBC (Thousand/cc)		14.14 ± 0.011	13.82 ± 0.013	14.13 ± 0.023	13.84 ±0.032	14.14 ±0.011	13.85 ± 0.040
Differential leukocyte (%)	Neutrophil	27.90 ± 0.025	30.68 ± 0.011	27.90 ± 0.022	30.71 ±0.012	27.84 ±0.023	30.71 ± 0.011
	Lymphocytes	63.54 ± 0.021	63.53 ± 0.022	63.54 ±0.044	63.53 ± 0.050	63.52 ±0.030	63.51 ±0.011
	Monocytes	1.28 ± 0.011	1.62 ± 0.031	1.30 ±0.024	1.60 ± 0.021	1.27 ±0.002	1.59 ±0.040
	Eosinophil	2.68 ±0.024	2.32 ±0.012	2.67 ± 0.044	2.32 ± 0.026	2.63 ±0.027	2.30 ± 0.012
Haemoglobin (%)		11.48 ± 0.016	11.70 ± 0.013	11.44 ± 0.022	11.72 ± 0.020	11.44 ±0.021	11.70 ± 0.012

C = Control Rabbits **; T_1 =** Rabbits treated with herbal gel (2.5 %); **T_2 =** Rabbits treated with herbal cream (5.0%); M_1, M_2, and M_3 = Sample mean value, SD_1, SD_2 and SD_3 = Standard deviations of control and experimental groups respectively , N= number of rabbits. P> 0.05, Non significant.

Table 8: Effect of repeated application of herbal gel for 28 days on Biochemical parameters of rabbits

Biochemical Parameters	C_1, $M_1 \pm SD_1$, (N=3)		T_1, $M_2 \pm SD_2$, (N=3)		T_2, $M_3 \pm SD_3$, (N=3)	
	14th Day	28th Day	14th Day	28th Day	14th Day	28th Day
Blood Sugar (mg/dL)	49.51 ±0.021	48.84 ± 0.016	49.15 ±0.017	48.84 ±0.015	49.51 ±0.023	48.84 ± 0.023
Total Cholesterol (mg/dL)	93.90 ±0.021	90.62 ± 0.014	93.91 ±0.201	90.63 ±0.021	93.92 ±0.033	90.63 ±0.024
Creatinine (mg/dL)	1.16 ±0.000	1.11 ±0.010	1.15 ±0.005	1.11± 0.005	1.15 ±0.005	1.12 ±0.010
Urea (mg/dL)	42.43 ±0.010	41.20 ± 0.010	42.44 ± 0.005	41.20 ±0.005	42.44 ±0.005	41.21 ±0.005
Total Bilirubin (mg/dL)	0.456 ±0.000	0.475 ± 0.000	0.457 ± 0.000	0.45 ± 0.000	0.46 ± 0.0001	0.474 ±0.000
Direct Bilirubin (mg/dL)	0.085 ±0.000	0.085 ±0.0001	0.086 ± 0.0001	0.085 ±0.000	0.086 ±0.000	0.085 ±0.0001
Total Protein (gm/dL)	6.43 ±0.010	6.34 ±0.0057	6.43 ±0.0100	6.33 ±0.005	6.44 ±0.011	6.36 ±0.010
SGOT (IU/L)	47.75 ±0.010	47.54 ± 0.0100	47.74 ± 0.010	47.53 ±0.015	47.74 ±0.010	47.53 ±0.0057
SGPT (IU/L)	42.42 ±0.011	42.34 ± 0.015	42.41 ±0.010	42.35 ±0.004	42.41 ±0.013	42.36 ±0.016
Alkaline phosphatase	45.90 ±0.010	45.65 ± 0.0100	45.92 ±0.0057	45.67 0.010	45.93 ±0.015	45.68 ±0.010
Acid phosphatase	51.60 ±0.015	50.93 ± 0.0100	51.62 ±0.010	50.92 ±0.005	51.60 ±0.011	50.93 ± 0.015

C = Control Rabbits **; T_1 =** Rabbits treated with herbal gel (2.5 %); **T_2 =** Rabbits treated with herbal cream (5.0%); M_1, M_2, and M_3 = Sample mean value, SD_1, SD_2 and SD_3 = Standard deviations of control and experimental groups respectively , N= number of rabbits. P> 0.05, Non significant.

Hematological analysis

Hematological profiles of the experimental rabbits have studied after the repeated application of herbal cream daily for 28 days. No detectable changes have observed in the values of these parameters compared to that of the control groups. The results have tabulated in Table 7.

Biochemical Analysis

Biochemical parameters of blood such as blood sugar, total cholesterol, creatinine, urea, total and direct bilirubin, total protein, SGOT, SGPT, alkaline phophatase and acid phosphatase of both test and the control rabbits were determined to check the change of these parameters after application of the herbal creams. The results have tabulated in the Table 8.

Histopathology of the rabbit's heart, kidney, liver and skin were carried out and compared with the control group which show no abnormalities or any deformation of the organs which revealed the complete safe and non toxic nature of the herbal cream containing *Stevia* extract.

Human Skin test:

The results showed that the formulation was devoid of any primary skin irritation or sensation or erythema, or edema even after 48 hrs of application on the human skin. None of the volunteers show any skin reaction in open sun light.

DISCUSSION

Stevia (*Stevia rebaudiana* Bertoni) seems to show several biologic roles such as non-caloric sweetener, anti-obesity or antioxidant effect especially in animal studies[18, 19, 20]. Moreover, there are some reports on antimicrobial effect of *Stevia* extract or it hypoglycaemic effects[21, 22]. However, To best of our knowledge, there was no structured study of topical *Stevia* extract cream 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.

Physico chemical properties of the prepared formulations showed good spreadability and tube extrudability with proper viscosity which is needed for cream based formulations. The undisturbed drug peaks in HPLC reports revealed that there are no drug excipient interactions in the formulations when compared with standard sample. The prepared formulations were found to be stable for six months and free from skin irritation on application to the effected part of rabbits. The present study revealed the potency of *Stevia* vanishing cream 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.

CONCLUSIONS

Our present study it concludes that the vanishing cream based *Stevia* white extract formulations will be useful for the treatment of dried skin when experimented on lab animals and human skin. The prepared vanishing cream was pleasant, effective, easily washable thereby increases the patient compliance. Therefore, it may be concluded that the test medication, moisturizer *Stevia* cream is safe and efficacious for human use. However, further structured study, especially in human phase, would be beneficial to assess its usefulness more exactly. 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 datas, which shall further give strength to our herbal and cosmetic industries eminence in global market.

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