

# Wound healing potential of hydroponic stevia rebaudiana in rats

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## Abstract

Studies revealed that Stevia has been used throughout the world since ancient times for various purposes; for example, as a sweetener and a medicine. The present study was carried out to evaluate the wound healing potential of extract of hydroponic Stevia rebaudiana Bertoni in experimental rats. All experiments were conducted following standard procedures. The extract was administered orally in dose of 20 mg/ kg was used for evaluating the wound healing potential in excision wound model for 2 weeks. Betadine (10%) was used as standard. In conclusion, hydroponic Stevia leaf powder accelerated wound healing activity in rats.

## Introduction

Stevia rebaudiana (Bertoni) is a herbaceous perennial plant of the Asteraceae family, native to Paraguay (South America). Stevioside, the major sweetener present in leaf and stem tissues of stevia, was first seriously considered as a sugar substitute in the early 1970s by a Japanese consortium formed for the purpose of commercializing stevioside and stevia extracts [1]. Diterpene glycosides produced by stevia leaves are many times sweeter than sucrose. They can be utilized as a substitute to sucrose [2]; they are natural sources of non-caloric sweetener and alternatives to the synthetic sweetening agents that are now available to the diet conscious consumers. Eight diterpene glycosides with sweetening properties have been identified in leaf tissues of stevia. These are synthesized, at least in the initial stages, using the same pathway as gibberellic acid, an important plant hormone [3]. The four major sweeteners are stevioside, rebaudioside-A, rebaudioside-C and dulcoside-A. According to Kinghorn the sweetness of these compounds relative to sucrose are 210, 242, 30 and 30 times, respectively [4]. The two main glycosides are stevioside, traditionally 5-10% of the dry weight of the leaves, and rebaudioside-A 24%; these are the sweetest compounds. There are also other related compounds including minor glycosides, such as rebaudioside-B, rebaudioside-C (12%), rebaudioside- D, rebaudioside-E, rebaudioside-F, dulcoside-A, dulcoside-C and steviolbioside, as well as flavonoid glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils [5]. Among the components of stevia, one, called rebaudioside-A, is of particular interest because it has the most desirable flavour profile [6]. Stevioside traditionally makes up the majority of the sweetener (60-70% of the total glycosides content) and is assessed as being 270 times sweeter than sugar. Rebaudioside-A is usually present as 30-40% of total sweetener and has the sweetest taste, assessed as 400 times sweeter than sugar with no bitter aftertaste (licorice taste or lingering effect). The ratio of rebaudioside-A to stevioside is the accepted measure of sweetness quality; the more rebaudioside-A the better. If rebaudioside-A is present in equal quantities to stevioside, it appears that the aftertaste is eliminated. The minor glycosides are considered to be less sweet, 30-80 times sweeter than sugar [7]. The sweetening effect of these compounds is purely taste; they are undigested and

no part of the chemical is absorbed by the body. They are therefore of no nutritional value [8]. Various research data revealed that plants may worked as healing and regeneration of the tissue by multiple mechanisms. There are several reports stating that the extracts of several plants, used for wound healing properties [9,10]. Stevia rebaudiana Bertoni plant was originated from South America (Paraguay and Brazil), belongs to the family Asteraceae, claimed as a potent wound healing plant. Traditional uses and earlier reports have revealed, enhanced healing with less scarring of cuts, wounds, burns, acne, seborrhea, dermatitis, and psoriasis after topical application of aqueous Stevia extracts [11]. These are mainly comprised of stevioside, steviobioside, rebaudioside A, B, C, D, dulcoside A, B [12].

## Materials

Hydroponic Stevia rebaudiana Bertoni collected from **G.S.Davtyan Institute of Hydroponics Problems** (Armenia), was used as a test plant for the present study. The plant was authenticated by PhD L. Hovhannisyan and Dr. M. Babakhanyan, Principal Scientist, **G.S.Davtyan Institute of Hydroponics Problems** and the specimen copy was preserved in the herbarium. The plant sample (leaves) was collected and oven dried at 60°C for 6 h. The dried leaves were stored at 4°C and were used for the further process.

## Animals

Male albino rats weighing 200-230 g were used in wound healing model experiments. A total of 15 animals used in experiments. Stevia dose for the study was 20 mg/kg. All animals were observed for food consumption for 2 weeks.

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**Excision wound model**

The rats were anaesthetized prior to creation of the wounds. All the surgical interventions were carried out under sterile condition.

The animals were divided into 3 groups of 5 each and the following treatments were given once daily for 2 weeks. *Experiments were performed* at Orbeli Institute of Physiology, Neuroendocrine Relationships Lab (Figure 1 and Table 1).

Group I: Control (no treatment).

Group II: Standard and treated with Stevia leaf powder (20 mg/kg)

Group III: Standard *Betadine* 10 %

The measurements of the wound areas of the excision wound model were taken on 1st, 7th and 14th day following the initial wound using transparent paper and a permanent marker. The recorded wound areas were measured with graph paper. Progressive decrease in the wound size was monitored periodically. Wound closure time of the tissue were studied. The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound. In the excision wound model, granulation tissue formed on the wound was excised on the 14th postoperative day. There was a full thickness epidermal regeneration which covered completely the wound area. The epidermis was thick and disorganized, especially when compared with the adjacent normal skin (Figure 2 and Tables 2-5).



Figure 1. Excision model in rat

Table 1. Biochemical, chemical, technical and radioactive composition of Stevia leaves

Indices	Ararat valley		Foothills	Nagorno Karabagh Republic (NKR) (Khanabad Village)	Literature data
	Soil	Hydroponics			
Extractive agents , %	45,2-54,0	46,0-63,8	47,9-52,9	46,1-52,0	32,5-40,9
Stevioside , %	8,1-8,4	8,9-9,2	8,5-8,6	8,0-8,5	4,6-8,2
Nitrogen , %	3,5-4,2	3,7-4,6	3,4-3,9	3,5-4,3	
Proteins , %	22,4-26,2	23,1-28,7	21,2-24,3	21,8-26,8	
Carotene , mg %	64,3	66,1	68,4	65,4	
Chlorophyll a+b, mg%	119,11	143,1	131,2	124,4	
Vitamine "C" mg%	62,3	74,9	72,1	68,9	
Tanning agent , %	13,9	10,2	12,5	12,9	
Flavonoids , %	5,1	4,4	4,8	5,2	3,5
Essential oil , %	0,1	0,2	0,2	0,1	
<b>Endemic microelements, mg/100 g</b>					
.._.._ J	0,8	8,7	0,6	0,5	
.._.._ Zn	0,9	1,3	0,7	0,6	
.._.._ Ge	0,00012	not detected	0,0001	0,00016	not specified
<b>Toxic technical elements, mg/kg</b>					
.._.._ Pb	0,12	0,10	0,10	0,09	
.._.._ As	0,08	0,06	0,07	0,05	
.._.._ Cd	0,24	0,20	0,18	0,16	
.._.._ Hg	0,003	0,002	0,002	0,02	
Pesticides DDT, mg/kg	not detected	not detected	not detected	not detected	-
Aflatoxin B <sub>1</sub> , .._.._	.._.._	.._.._	.._.._	.._.._	
Radionuclides, U · 10 <sup>-6</sup> %	1,8	1,3	1,1	-	-
<sup>90</sup> Sr-Bq/kg	18,6	14,4	15,8	-	.._.._

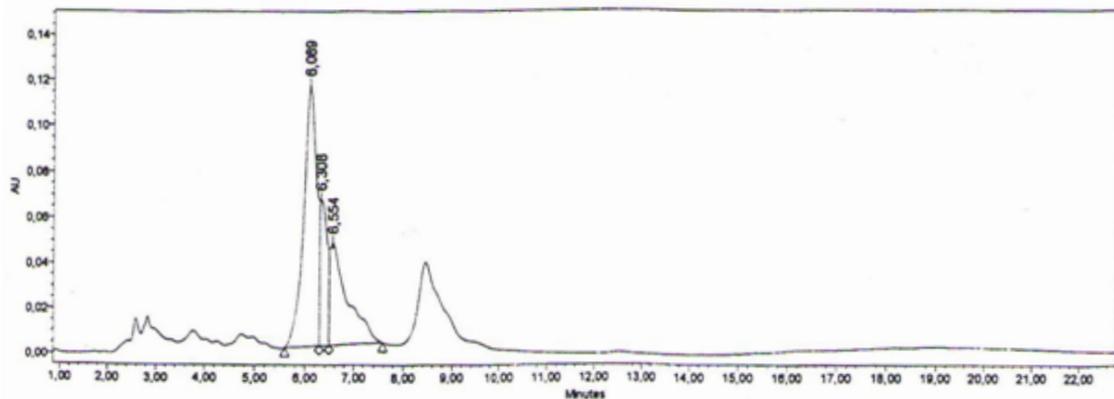


Figure 2. Thin layer chromatography of Stevia

Table 2. Content of biologically active substances in Stevia (sample 1, standart) (thin layer chromatography was used, Silica gel 60 F 254 (Merck-Germany)

N	Name	Retention Time	Area	% Area	Height
1	Standart	6,089	2061029	53,20	114769
2	Rebaudioside A	6,308	662200	17,09	64659
3	Rebaudioside C	6,554	1150654	29,70	45328

Table 3. Content of biologically active substances in Stevia (sample 2, soil)

N	Name	Retention Time	Area	% Area	Height
1	Stevioside	6,716	9377124	77,65	326455
2	Rebaudioside A	7,605	2698935	22,35	76589

Table 4. Content of biologically active substances in Stevia (sample 3, hydroponics)

N	Name	Retention Time	Area	% Area	Height
1	Stevioside	6,279	13554416	71,37	566682
2	Rebaudioside A	6,816	5438482	28,63	202741

Table 5. Content of biologically active substances in Stevia (sample 4, Nagorno Karabagh Republic, Khanabad Village). Contains diterpene glycosides about 83% and monosaccharides only 0.05%. The highest Rebaudioside A content was found in sample 4

N	Name	Retention Time	Area	% Area	Height
1	Stevioside	6,999	907785	79,01	30924
2	Rebaudioside A	7,714	241102	20,99	7809

## Discussion

Wound healing is a complex process of restoring cellular structures and tissue layers in damaged tissue together to its normal state and commencing in the fibroblastic stage where the area of the wound undergoes shrinkage [13]. It comprises of different phases such as contraction, granulation, epithelization and collagenation [14,15]. Wound healing can be discussed in three phases viz. Inflammatory phase, proliferative phase and maturational or remodeling phase. The 20 mg/kg Stevia was recorded similar effectiveness when compared to the control group treated with a *Betadine* (10%) (Figure 1). Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Studies were revealed that flavonoids are also known to promote the wound healing process mainly due to their astringent and antimicrobial properties which appear to be responsible for the wound healing and increased rate of epithelialization [16]. So the study provides a rationale for the use of hydroponic Stevia preparations in the traditional system of medicine to promote wound healing. This effect may be explained by several mechanisms such as coating the wound. Further the Stevia

leaf powder did not produce any adverse effect and because of this it is possible to recommend its use in the treatment of wounds.

## Conclusion

The study thus demonstrated the wound healing activity of hydroponic Stevia leaf powder and found to be effective in the functional recovery of the wound healing.

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