Is there enough information to determine the toxicity of "stevia" and its sweeteners compounds?

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INTRODUCTION

Every human being needs good nutrition to develop mentally, physically and physiologically.

This improves their quality of life and allows them to be more productive at work, contributing to the general betterment of life in society. In the past two decades scientists have worked on finding non-nutritive sweeteners to substitute the "Sweet-"flavor, capitalizing the human weakness for the consumption of various which is causing serious health problems. Currently, as part of the trends of body health and caring for the figure according to beauty stereotypes, good nutrition is been encouraged and often associated to the consumption of sweeteners of natural origins.The excessive consumption of sugars and sedentary lifestyles been seen as the possible cause of varios diseases. Thus, consumers are lookin for a sweetener with acceptable taste that doesn't have negative effects on health, and, *Stevia rebaudiana* seems to be an alternative solution. It's sweet component (-steviol glycoside), has several advantages: it can be used leaves or refined, the sweetener contains no calories, has a mild aftertaste or bitterness, has good durability and the extracts, steviol glycoside, are 300 times sweeter than sugar.

According to several authors, *Stevia rebaudiana* (Bertoni), is a perennial shrub of the sunflower family Asteraceae, native to Paraguay and Brazil. It is also known as "the sweet"-grass of Paraguay". Stevioside, is the main sweet component in its leves.

The Stevia was first studied by Pedro Jaime Esteve (1500 – 1556). The plant was already used in pre-Columbian times by the Guarani tribe and one of its species, Stevia rebaudiana, was known as "sweet herb". The identification was made by Moisé Santiago Bertoni in 1887 who also discovered sweetening properties. In 1905, was registered as Stevia rebaudiana Bertoni in international books. In 1959, studies began various laboratories in Brazil and the world. In 1966, Paraguay began selling "Kaa-Hee" leaves and, by this times, the investigation of Stevia in many countries around the world intensified. In Japan, important advances were achieved; all active ingredients were studied, its safety was tested and the Japanese industry began its mass processing, which continues favorably to the present.

In the United States, Japan, Brazil, Argentina, Colombia, China, India, Australia and Thailand, among other countries, the plant is massively used with great success and without any contraindications.

The plant Stevia and stevioside extracts have been used for years as a sweetener in South America, Asia, Japan, China and different countries of the European Union. Since 1995, in the United States, Stevia is used as a dietary supplement. In 2000, the European Commission refused to accept it as a new food motivated to a lack of scientific reports and discrepancies between studies regarding possible toxicological effects of stevioside and steviol aglycone. (Kinghorn , 2002)

According to several authors Stevia has shown promising results in medical research with various beneficial effects, hypoglycemic properties, vasodilating, diuretic and cardiotonic, antacid properties, oral antibacterial, and digestive, among others. Since it is considered that Stevia has pharmacological or biological activity, because of the expression of beneficial or adverse effects on living matter. The focal point of this study is to determine if: there is enough information to prove the toxicity of "Stevia" and its sweetening compounds?.

So far it has not been determinated if Stevia or its companents have any toxic effects. Its; leaves are the ones that have the sweetening power and these can be eaten fresh, dried, in infusion, crushed or as an ingredient in food. In 2006, the available evidence on the safety assessment published by World Health Organization (WHO) found no adverse effects of steviol glycosides. There is no conclusive evidence that Stevia presents any potential risks to human health.

The main class of biological activity is the toxicity of the substance. This activity is generally dose-dependent and is not common to have effects on a range of beneficial to adverse for one substance when going from low to high doses. The activity is critically dependent on the fulfillment of the criteria of absorption, distribution, metabolism and excretion (ADME).

METHODOLOGY

To research consisted in a documental study. It was based on a general search for articles related to our object of study. Bibliographic data collection was quantified from the website specialized "PubMed" using the following phrase: "Stevia OR steviol". On May 4 came a result of 408 research articles.

We proceeded to make a document listing the items and placing their title, references and abstract. This document was the basis for classifying them into different categories according to the type of research conducted, as follows: 1) Chemicals (Q) , studies related to the extraction, purification, separation, quantification, identification of compounds including chemical treatment processes Stevia sweeteners and their compounds; 2) Benefits (B) , studies carried out to highlight the beneficial properties of consumption, use of Stevia sweetener or its components on human health ; 3) Toxicology (T), studies researching the toxicity of Stevia and its sweetener compounds on different levels or types of toxicity; 4) Botanical / physiological (BF) , includes studies aimed at a better plant development, different forms of cultivating it, plant physiology and 5) Other (O) research encompasses all those studies that did not belong to any of the previous categories; aiming to be informative about what is Stevia, its use as drugs; cover, and in food additives, among others.

Subsequently, it was cataloged and the number of items was quantified per year from 1965 to 2014 annexing the total studies per year per category. Percentage charts were drawn for the quantitative comparison of the number of studies published on the Web "PubMed" page on Stevia sweeteners and compounds from 1964 to the present day (50 years), classified by categories. Moreover, an informative table was made with the items classified as Toxicological, to what was applied and whether the results toxicity were positive or negative. From the obtained results, though the analysis of the tables and graphs we arrived to relevant conclusions from the documental review.

RESULTS AND DISCUSSION

Table 1. Categorical studies quantification in a period time from 1965 to 2014

Year	B	T	C	BP	O	total per year	Year	B	T	C	BP	O	Total per year
1965	0	0	2	0	0	2	1990	0	0	0	1	0	1
1966	0	0	0	1	0	1	1991	0	0	5	0	3	8
1967	0	0	0	0	0	0	1992	0	2	0	0	3	5
1968	0	0	0	0	1	1	1993	0	1	0	1	0	2
1969	0	0	0	0	0	0	1994	1	1	1	1	0	4
1970	0	0	0	0	0	0	1995	1	1	1	0	1	4
1971	0	0	0	0	0	0	1996	1	2	3	0	0	6
1972	0	0	0	0	0	0	1997	0	3	0	0	2	5
1973	0	0	0	0	0	0	1998	1	1	0	1	0	3
1974	0	0	0	0	0	0	1999	0	2	1	0	2	5
1975	0	0	0	2	0	2	2000	0	0	6	0	2	8
1976	0	0	1	0	0	1	2001	2	1	1	0	2	6
1977	0	0	0	2	1	3	2002	3	3	2	1	2	11
1978	0	0	0	1	0	1	2003	4	1	2	0	5	12
1979	0	0	0	0	0	0	2004	6	1	2	0	2	11
1980	1	0	0	0	0	1	2005	4	0	5	0	3	12
1981	0	0	0	0	1	1	2006	4	2	4	0	3	13
1982	0	0	0	0	1	1	2007	1	2	4	0	2	9
1983	0	0	2	0	0	2	2008	4	5	6	2	6	23
1984	0	0	1	2	1	4	2009	5	1	12	1	3	22
1985	0	1	0	0	3	4	2010	2	0	5	2	3	12
1986	0	2	1	1	1	5	2011	4	0	19	1	6	30
1987	0	0	1	0	3	4	2012	6	0	15	1	9	31
1988	0	0	1	2	0	3	2013	9	2	14	11	10	46
1989	0	2	0	0	0	2	2014	11	0	9	4	11	35
B=70, T=36, C=126, BP=38, O=92, n=362													

Table 1. Quantification number of studies published on the Website PudMed about – Stevia's sweeteners and their compounds in the period between 1965 to the present divided into categories: B, T, C, BP and O.

Table 1 and Graph 1 show the quantification and percentage value of the results obtained from the collection of bibliographic and documentary research of various media related to the object of study.

Chemical studies represent the highest percentage with 35%. They show a great interest in the extraction, purification, and identification of compounds including chemical treatment procedures of Stevia and its sweetener compounds. Possibly motivated by the growing global trade in sugar substitutes and the idea that the consumption of natural products is healthy.

Followed by O, with 25% concerned with what is Stevia, its use drugs as covers, use as additives in foods and their medicinal properties. The B studies, 19% oriented towards the benefits of consuming Stevia or its sweeteners components. The BP represent 11%, research studies on how to improve the development, forms of cultivating and the physiology of Stevia.

From the 362 articles published on the PubMed Web site on the toxicity of Stevia sweeteners and their compounds in the last 50 years, only 36 were investigated, which represents a 10%. Perhaps it hasn't been given enough importance in order to have greater disclosure on the cases exposed above.

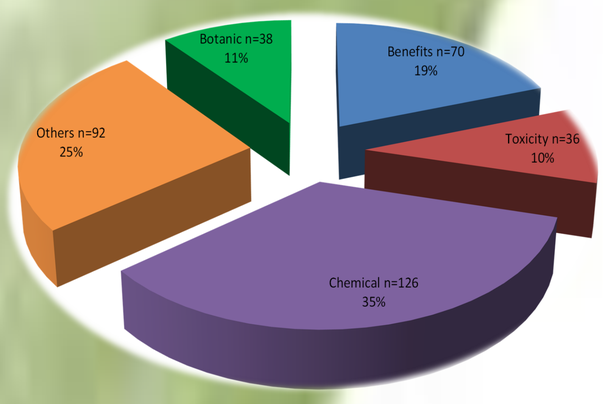
In Table 1, we see that in the years 1967, from 1969 to 1974 and in 1979, no studies were conducted on Stevia sweeteners or their components. There was also an unstable behavior, increases and decreases, from 1982-1998 and then an increase is initiated through 2013 with the exception of the years 2007, 2010 and 2014.

It was noted that years 2002, 2008, 2009 and 2013 are the only ones with studies from all categories. The rest of the years only 2 or 3 types of studies were carried out per year on the subject.

The first study about the beneficial properties was conducted in 1980, then another from 1994 to 1996, 1998 to 2001. An in crease in the number of studies per year to the present can be observed.

Toxicological studies were initiated in 1985, continued intermittently in the years 1986, 1989, 1992, until 2009. They began with an average of 2 studies per year except 2000 and 2005. The latest ones were registered in 2013.

Chemical studies were first conducted in 1965, 1976. From 1983 they began to be continued annually until the present, except only for seven years during that entire period of 31 years.



Graph 1. Percentage representation of published studies on the Website PubMed about Stevia's sweeteners and their compounds during 50 years, classified into categories: benefits, toxicological, chemical, botanical / physiological and others.

Botanical / Physiological studies are those with the largest distribution between the study period of 50 years. Stands out that for the year 2013 11 of these type of studies were carried out, which differs markedly from the behavior of the other years that had conducted a maximum of 2 studies per year.

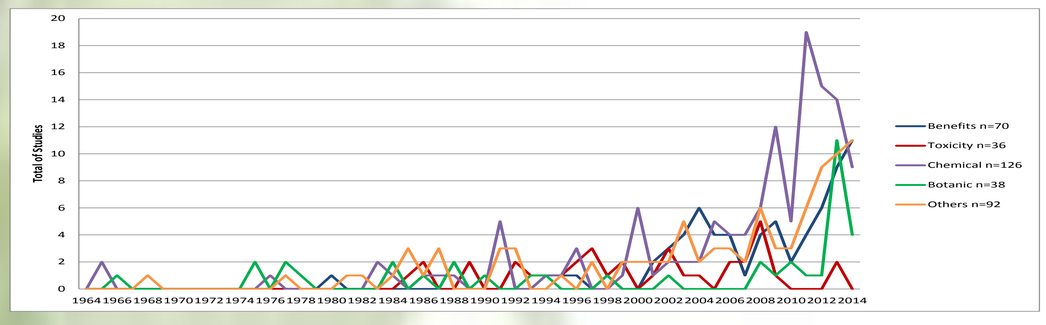
The studies classified as 'others' have erratic trends until 1999 where they stabilized with an increasing trend until now.

In the Graph 2. Over the entire period there's a trend in the categories of studies starting from 1965. In general, by 1985 all categories present an average of 3 studies per year with certain peaks in chemical studies, reaching 5 and 6 studies per year, but again returning to the average.

By the year 2002 the number of studies by year tend to increase in descending order: as chemical, other, beneficial and botanical / physiological. Toxicological studies are the only ones with a reduced tendency to present.

Table 2. The category referred to as Toxicological, being the main purpose of this study, was classified by type of studies in order to ascertain what information was provided by these publications. To that end, it was determined how the study was carried out (on what) and if the described effects were considered as toxic or non-toxic according to the research.

It was observed that the most common toxicology studies were of the toxicokinetic type: 15 out of the 36 identified studies. In general, the studies were based on *in vivo* & *in vitro* testing of animals, primarily rats, of their urinary excretion and renal uptake, followed up by studies of the functions of absorption-metabolism- excretion of carbohydrate. These type of test was conducted only once with people.



Graph 2. Representation of the quantification number of studies published on the Website PudMed about – Stevia's sweeteners and their compounds in the period between 1964 to the present (50 years) divided into categories: benefits, toxicological, chemical, botanical / physiological and others.

The second most common type of study was of the Mutagenic type, with a total of 7 items. All of these were conducted in vitro with *Salmonella typhimurium* strains. In these studies there were contradictions between the obtained results on the mutation of *Salmonella typhimurium steviol* since metabolic activation in the case of references 14 and 32 was negative but in references 16 and 12 was positive. Thus, despite been similar studies they reached different and contradictory results and conclusions.

Genotoxicity studies also showed conflicting results. The three were conducted in vitro from which two used comet assay. In reference 26 toxicity tested positive but negative in reference 32. The third example also tested positive for toxicity.

Of the type of toxicological studies on embryology/reproduction three were quantified and the reference article 39 contradicts the other 2 references numbered 31 and 8. According to the first the substance has toxic effects on embryos and pregnancy, whereas the latter 2 indicated the contrary. Although the three studies were conducted with different animals species, hamsters and mice, they all belong to the same family.

In both cytotoxic studies it was determined that Stevia and its components were toxic to cancer cells, both studies were conducted in vitro. Once again, there were contradictions in the results of the different studies. In this case regarding the results on chronic toxicity; which tested positive for toxicity in one study but not the other. (References 27 and 20).

In both articles dealing with subchronic toxicity it was found that, in vivo, there was a negative response from the rats. (Reference 11 and 5).

Table 2. Toxicology studies classification finde in a period time from 1964 to 2014

Year	Study Type	Applicator	Effect	T	Ref.	Year	Study Type	Applicator	Effect	T	Ref.
1985	Toxicokinetics	in vivo	inhibited oxidative phosphorylation, ATPase activity NADH-oxidase activity, succinate-oxidase activity, succinate dehydrogenase, and L-glutamate dehydrogenase.	yes	10	2001	Toxicokinetics	in vitro.	An inhibitory effect of steviol on transepithelial transport of p-aminohippurate	yes	3
1986	Mutagenicity	in vitro-bacterial	test systems, the aglycone, steviol (13-hydroxy-ent-kaurenoic acid and potential inducers of glutathione S-transferase activity in rats	yes	29	2002	Genotoxicity	in vitro - in vivo	DNA damage in multiple mouse organs (comet assay)	no	32
1986	Toxicokinetics	adult human	leaves on a glucose tolerance test	yes	4	2002	Mutagenicity	in vitro m.o.	mutation assay - Salmonella typhimurium TM677 with metabolic	yes	34
1989	Mutagenicity	in vitro m.o.	mutation assay using Salmonella typhimurium TM677 with or without metabolic activation	no	15	2002	Subchronic toxicity	animales-ratas-3meses	weight gain, visual inspection hematologic and chemical analysis	no	11
1989	Chronic toxicity	in vivo-rats-60 days	endocrine parameters of male rat	no	27	2003	embriotoxicity	broiler embryos	prenatal exposure to stevioside and steviol	no	8
1992	Carcinogenicity	in vivo	S. sobrinus counts were made and caries was evaluated according to Keyes' technique.	no	6	2004	Cytotoxic	human cell	cancer and human embryonic lung cells	yes	14
1992	Toxicokinetics	hypertension rats	renal function	yes	23	2006	Reproduction toxicity	female rats 60 day	Reproductive disturbances, number of corpora lutea, dead fetuses and implanted	no	31
1993	Mutagenecity	in vitro m.o. human cell	mutagenicity in Salmonella typhimurium strains TA98 and TA100 and for chromosomal effects on cultured human lymphocytes.	no	33	2006	Toxicokinetics	animals-oral-15day	Inhibition of the hepatic gluconeogenesis - glycemic	no	7
1994	Toxicokinetics	animal-oral-fasted rats	hepatic glycogen synthesis	yes	9	2007	Genotoxicity	Eukaryotic cells	the DNA-induced damage was evaluated using the single cell gel electrophoresis (comet assay)	yes	26
1995	Toxicokinetics	in vitro	intestinal glucose absorption	yes	36	2007	Mutagenicity	in vitro- the strain TA98	antimutagenic effect of methanolic extracts obtained from leaves, root, and flowers	yes	2
1996	Genotoxicity	in vitro - in vivo	mutagenicity tests using bacteria	yes	16	2008	Subchronic toxicity	in vivo-rats-30 dies	The NOAEL, evaluation of testes histopathology	no	5
1996	Mutagenicity	in vitro m.o.	mutations induced by metabolically-activated steviol, induces mutations at the guanine phosphoribosyltransferase gene (gpt) of Salmonella typhimurium TM677	yes	17	2008	Toxicokinetics	animals-oral-broiler	effects on feed intake, nutrient metabolism, blood parameters and growth performance	yes	1
1997	Mutagenicity	in vitro m.o.	mutations induced by metabolically-activated steviol, of Salmonella typhimurium TM677	yes	12	2008	Toxicokinetics	animals	absorption, distribution, metabolism and excretion compounds	no	30
1997	Acute toxicity	animals-hamster	Histopathological examination, cause and mortality	yes	35	2008	Toxicokinetics	chronic consumption humans	glucose homeostasis, blood pressure in individuals with type 2 diabetes mellitus	no	18
1997	Toxicokinetics	in vivo -rats-15days	renal function	yes	22	2008	Toxicokinetics	in vivo -rats	general condition and behavior, hematology, weigh	no	25
1998	Toxicokinetics/ embriotoxicity	pregnant hamster / embryos	embryo development and pregnancy	yes	39	2009	Toxicokinetics	animals-oral-rats	Renal excretion of esteviol	yes	19
1999	Chronic toxicity	male rats -60 day	final weight of testis, seminal vesicle and cauda epididymidis	yes	20	2013	Cytotoxic	in vitro - leukemia, lung, stomach, and breast cancer cell lines	typical apoptotic cell de	yes	37
1999	Toxicokinetics	male rats	renal water, Na+ and K+ excretion	yes	21	2013	Toxicokinetics	in vitro - animals	metabolism in vitro de matrices- simulated gastrointestinal fluids, rat liver microsomes, and rat cecal contents	no	24

Table 2. Representation of toxicological studies type listed on the PubMed Web page about Stevia's sweeteners and compounds from 1964 to the present (50 years), their application, effect and possible toxicity.

Only One carcinogenic study was identified and it presented a negative toxicological response (Reference 6).

Finally of the type of study on acute toxicity, there was only one, published in 1997, with a positive result on toxicity. The possible cause of death induced by steviol could be associated to acute renal failure. It is important to consider this result since the toxicokinetic studies about renal function tested positive for toxicity in the electrolyte excretion (References 21, 22 and 23). Seeming, thus, a potential source of indirect toxicity that can lead to death in extreme cases.

CONCLUSIONS

After analyzing the collected data it can be concluded that the material obtained through the various examined articles, and PubMed Web site about the toxicity of Stevia sweeteners and its components is insufficient and inconsistent

Toxicity studies only represent 10% (36 items) of the 362 articles published in the PubMed website. In the examined period of 50 years, there are several years in which no studies on the subject were conducted. Perhaps these type of studies have not received enough attention due to the focus, on its natural and beneficial properties. Some of the published studies, with similar methodology and application trials presented conflicting results regarding toxicity. Therefore, more research is needed to determine whether or not Stevia and its sweetener components are potentially toxic and, at what level of exposure could they have adverse effects on human health.

It is noteworthy that there's only one acute toxicity study when it presented deadly effects by generating kidney problems, which could mean that Stevia is potentially toxic and harmful to health and requires, thus, further investigation into the case. Furthermore, this potential problem is reaffirmed with the results of the toxicokinetic studies on renal function, where electrolyte excretion tested positive in toxicity (References 21, 22 and 23 of Table 2).

It was concluded that in the case of Stevia and its sweetener compounds there seems to be a greater interest in the study of the extraction, purification, separation, quantification and identification of compounds from other chemical processes, rather than its toxicity. Possibly, this has something to do with the growing global market for sugar substitutes, with applications in the food and pharmaceutical industry, and the growing commercialization and consumption of natural products, Stevia being a combination of both things, a non-caloric natural product. Moreover, the ancient cultural beliefs in the benefits of its consumption now linked to the circulation of supposedly healing and healthy properties, in some cases not proven, also contribute to deflect the research.

It is recommended that further toxicological studies are conducted to check the actual effects of Stevia and its active compounds on human health, whether this prove beneficial or toxic..

Thanks to God, my parents, my brother, my husband for always serving me as a support to fulfill this stage of my life; my Tutor Prof (a) Eva Castells, for having spent some of your valuable time, by orienting and guiding the development of this work to grade; the teachers and all the university staff for their dedication and skills provided and to all the people who in one way or another helped me.