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EFFECT ON EXTRACTS OF *STEVIA REBAUDIANA* BERTONI. IN ETHANOL INDUCED GASTRIC ULCER BY USING WISTER RATS

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Abstract

The sweet herb of Paraguay, *Stevia rebaudiana* Bertoni produces, in its leaves, just such an alternative with the added advantage that *Stevia* sweeteners are natural plant products. In addition, the sweet steviol glycosides have functional and sensory properties superior to those of many other high potency sweeteners. The PESR, EESR & AESR has investigated the anti ulcerogenic activity by using Wister rats in both sexes. Oral administration of petroleum ether, ethanol & aqueous extracts of *S. rebaudiana* (100 & 300 mg, p.o) produced a significant (p<0.01) and dose dependent inhibition to the acute ulcer induced by Ethanol 99% of 2.5 ml/kg/body weight at once to rats and the parameters of gastric secretion were evaluated. When compared with cimetidine (100 mg/kg, p.o) volume, pH and free acidity of gastric juice in ulcerogenic property, *S. rebaudiana* showed the significant activity in 300 mg/kg/body weight <p0.01. They exhibited a significant (p<0.01) inhibition of gastric lesions by decreased the free acidity and the ulcer index. The inhibitory effect of the *S. rebaudiana* on lesions induced by stress was compared to that of cimetidine. The volume and concentration of Gastric juice were increased after oral administration of the AESR. A significant reduction of the lesion index was observed in the acute assays. An effective significant alteration in all biochemical and histopathological parameters was investigated. From this we can conclude that the *S. rebaudiana* having the potential effectiveness at the dose of 300 mg/kg/body weight, <p0.01 by dose dependent manner. These results suggest that leaves of *S. rebaudiana* having the cytoprotective property, which support the antiulcer effect of this plant in the traditional medicine.

Key Words: Anti-ulcer; *Stevia rebaudiana*; Steviol; Stevioside; Steviolmonoside; Stigmasterol.

Introduction

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood through the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for various diseases, for which no scientific proof is available. One such plant, *S. rebaudiana* Bertoni is one of 240 members of the genus *Stevia*. *Stevia* is a genus of about 240 species of herbs and shrubs in the sunflower family (*Asteraceae*), native to subtropical and tropical South America and Central America. The species *S. rebaudiana* Bertoni, commonly known as sweet leaf, sugar leaf or simply *Stevia*, is widely grown for its sweet leaves. As a sugar substitute, *Stevia*'s taste has a slower onset and longer duration than that of sugar, although some of its extracts may have a bitter or licorice-like aftertaste at high concentrations. For centuries, the Guaraní tribes of Paraguay and Brazil used *Stevia*, which they called ka'a he'ê ("sweet herb"), as a sweetener in yerba mate and medicinal teas for treating heart burning and other ailments (Tanvir, 2005). More recent medical research has shown promise in treating obesity, high blood pressure, and hypertension. *Stevia* has a negligible effect on blood glucose, even enhancing glucose tolerance (Curi, 1986); therefore, it is attractive as a natural sweetener to diabetics and others on carbohydrate-controlled diets (Gregersen, 2004). Presently an attempt is made to evaluate its anti ulcer activity of ethanol extract of *S. rebaudiana* Bert. Ulcer is a major problem in

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this prevalence technological world. An ulcer (from *Latin ulcus*) is an open sore of the skin, eyes or mucous membrane, often caused by an initial abrasion and generally maintained by an inflammation and/or an infection. In other words, it is a macroscopic discontinuity of the normal epithelium (microscopic discontinuity of epithelium is called erosion). Ulcer is a systemic chronic disease affecting multiple organs but gastric ulcer specific to the stomach having ulcerous lines. Ulcers are non-healing wounds that develop on the skin, mucous membranes or eye. Although they have many causes, they are marked by loss of integrity of the area, secondary infection of the site by bacteria, fungus or virus, generalized weakness of the patient, lengthy healing time. The ulcer having the different stages as in the first stage skin redness and underlying tissue soft. The redness disappears with minor pressure. In second stage there is redness, swelling and hardening of the skin around the area. Sometimes there is blistering whether loss of the superficial skin. In third stage skin becomes necrotic. There may be exposure of the fat beneath the skin. The skin may be lost through all its layers. In fourth stage is more loss of fat and more necrosis of the skin through to the muscle beneath. In the fifth stage continued loss of fat and necrosis of muscle cells. In the final stage bone destruction begins with irritation of the bone, erosion of the bone cortex progressing to osteomyelitis. There may be sepsis of a joint, pathologic fracture or generalized body infection, septicemia. Ulcer affecting various organs and area in human beings that are stomach ulcer, peptic ulcer, pressure ulcer (decubitus), peptic ulcer, crural ulcer, cushing ulcer, curling ulcer and hunnars ulcer.

Plant Description

S. rebaudiana is a small perennial growing up to 65-80 cm tall, with sessile, oppositely arranged leaves. Different species of *Stevia* contain several potential sweetening compounds, with *S. rebaudiana* being the sweetest of all. *Stevia* is a semi-humid subtropical plant that can be grown easily like any other vegetable crop even in the kitchen garden. The soil should be in the pH range of 6.5- 7.5; well-drained red soil and sandy loam soil. Saline soils should be avoided to cultivate this plant (Melis, 1996).

Materials Methods

The *S. rebaudiana* leaves collected from Mukombu, Tiruchirappalli and surrounding area. The leaves were dried at room temperature without any moisture content. After 10 days and coarsely powdered with the help of a hand grinding mill and the powdered plant material was passed through sieve number 40. The coarse powder was extracted separately by continuous hot extraction process using soxhlet apparatus (Harborne, 1984) with different solvents successively in increasing order of polarity from petroleum ether, ethanol and finally fresh aqueous extract (Kokate, 1994)

Figure 1: *S. rebaudiana* Leaves with Flowers

Figure 2: *S. rebaudiana* with Stem

Classification:

Kingdom	: *Plantae*
Division	: *Magnoliophyta*
Class	: *Magnoliopsida*
Order	: *Asterales*
Family	: *Asteraceae*
Tribe	: *Eupatorieae*
Genus	: *Stevia*

Experimental Animals

Wistar albino rats of either sex and of approximately same age procured from Laboratory at Animal Resource Section, Laboratory medicine, Madharavam, Chennai were used in this study. Weighing about 150-250 g was used for each group. Each group consisted of 6 animals. They were housed in polypropylene cages and fed with standard diet and *water libitum*. The animals were exposed to alternate cycle of 12 hour light/ 12 hour dark and 25$\pm$ 3^{o}C, 35 – 60% humidity. The experimental protocols were subjected to the scrutinization of the Institutional Ethical Committee and cleared by the same.

Acute Toxicity Studies

The acute oral toxicity study was carried out as per OECD guidelines. The LD_{50} cut-off dose was found to be 5000 mg/kg body weight for aqueous extract of AESR. They were not produced any sign of toxic to animals. The animals were shown the normal activities on behavior. (OECD Guideline, Oct-2000)

Phytochemical tests

The dry extracts leaves of *S. rebaudiana* were separately tested for the presence of flavonoids, glycosides, saponins, tannins and alkaloids (Dahou, 2003).

Drugs and chemicals

Anti Ulcer activity

Wistar albino rats of either sex were allotted into different groups fasted for 24 h prior receiving an oral dose of saline (NaCl 9‰, 5 ml/kg). Animals were randomly divided into eight groups of six animals each (n=6). First group used as positive control received After 30 min, all groups were orally treated with 1ml of 150mM HCl/EtOH (40:60, v/v) solution for gastric ulcer induction. The second group received gastric lesions with standard drug as cimetidine (100 mg/kg, p.o) as reference

compounds. The third, fourth, fifth, sixth, seventh and eighth group were received anti-ulcerogenic activity of petroleum ether extract of *S. rebaudiana* (PESR), ethanol extract of *S. rebaudiana* (EESR) and aqueous extract of *S. rebaudiana* (AESR) (100 and 300 mg/kg, p.o) respectively, derived from *S. rebaudiana* was studied in 150mM HCl/EtOH induced gastric ulcer (Hara, 1985). Animals were killed 1 h after the administration of ulcerogenic agent; their stomachs were excised and opened along the great curvature, washed and stretched on cork plates. The surface was examined for the presence of lesions and the extent of the lesions was measured. The summative length of the lesions along the stomach was recorded (mm) as lesion index (Borgi, 2007).

Gastric secretion parameters

Gastric secretion volume, pH and HCl concentration were measured according to the method of (Shay, 1945). All groups of rats were fasted 24h, with free access to water. Immediately after pylorus ligature, NaCl 9‰ (5 mg/kg), different *S. rebaudiana* extracts cimetidine (100 mg/kg) and were administered orally. After 4 h, animals were killed by cervical dislocation, the abdomens were opened, the stomachs were removed and the gastric content was collected to determine the total amount of gastric juice (ml) and pH values. Total acidity, free acidity was checked in gastric secretion was determined by titration to pH 7.0 with 0.1N NaOH (Shay, 1945).

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan t' test for multiple comparisons. The table was analyzed by using Dunnet t' test with prism software. The significance of difference was accepted at $p<0.01$. (Kulkarni, 1993)

Table 1. Effect of various extracts of *S. rebaudiana* Bert, leaves in ulcer induced rat stomach.

Groups / Parameters	Dosage (mg/kg)	Free Acidity (Equiv./100 g/4 h)	Total Acidity (Equiv./100 g/4 h)	Gastric Juice (ml)	pH (units)	Ulcer Index (mm)
Ulcer Control (Ethanol induced)	2.5 ml	77.88±2.09***	98.1±0.96***	4.46±0.41'	3.55±0.51'	58.08±7.06***
Standard Control (Cimitidine)	10	17.53±1.13***	26.21±3.64***	2.38±0.32'	5.35±0.31***	21.54±3.60***
PESR	100	19.1±3.42***	29.23±1.98***	3.44±0.42'	5.21±0.19'	33.22±2.57***
PESR	300	16.15±2.47***	23.1±2.40***	3.16±0.25'	5.77±0.30***	29.25±1.66***
EESR	100	24.25±2.54***	24.81±1.32***	1.70±0.42'	4.40±0.47***	28.95±3.85***
EESR	300	23.28±3.64***	19.88±2.76***	1.35±0.26'	4.83±0.41***	25.29±2.06***
AESR	100	31.85±2.89***	25.48±1.84***	4.32±0.32'	3.3±0.27'	13.53±0.46***
AESR	300	26.2±3.08***	21.16±1.53***	3.69±0.59'	3.9±1.10'	2.73±0.72'

Standard control shows, F=999.31***, $p<0.01$, Cimitidine, F=115.21***, $p<0.01$,
PESR 100 mg/kg, F=244.07***, $p<0.01$, PESR 300 mg/kg, F=249.37***, $p<0.01$,
EESR 100 mg/kg, F=207.25***, $p<0.01$, EESR 300 mg/kg, F=143.88***, $p<0.01$,
AESR 100 mg/kg, F=397.93***, $p<0.01$, AESR 300 mg/kg, F=272.44***, $p<0.01$,

Figure 3. Anti-Ulcer activity of PESR, EESR & AESR

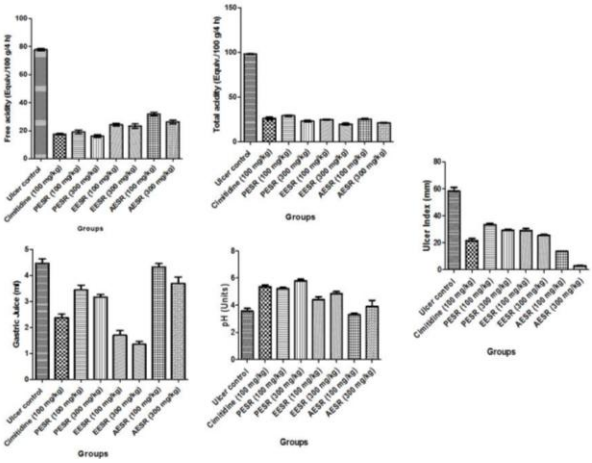
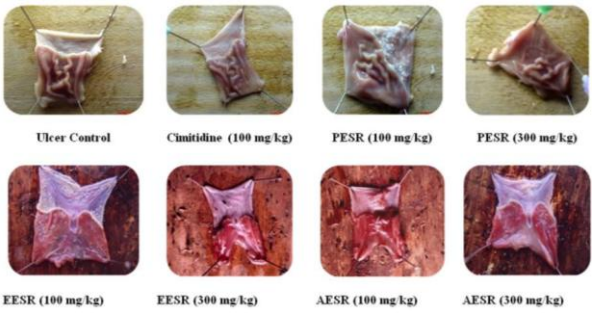


Figure 4. Morphological characteristics of the Stomach ulcer

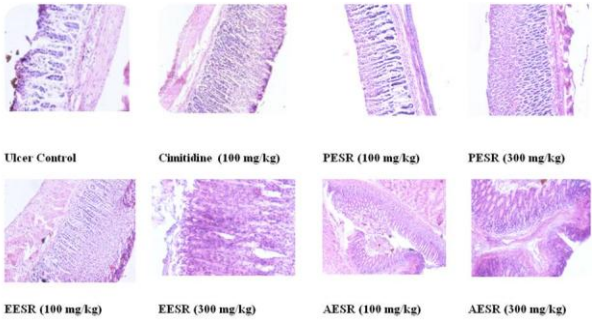


Result and Discussion

Ethanol serves as a most common ulcerogenic agent and when given intragastrically to rats it produces severe gastric hemorrhagic erosions. The genesis of ethanol-induced gastric lesions is multifactorial with the depletion of gastric wall mucus content as one of the involved factors and this damage induced by ethanol may be due to mucosal leukotriene release (Al-Harbi, 1997). Ethanol-induced damage to the gastric mucosa is associated with a significant production of free radicals leading to an increased lipid peroxidation and damage to the cell and cell membranes. Accumulation of activated neutrophils in the gastric mucosa may be a source of free radicals *(*Al-Harbi, 1997). Ethanol treatment caused a significant increase in the ulcer index whereas in *S. rebaudiana post* treated *rats;* there was a significant reduction in the ethanol effect by two different concentrations with three different extracts. There are extensive experimental evidences which indicate that free radical scavengers protect the gastric mucosa. The reduction in the concentrations of ulcer index of the stomach in the ethanol-induced rats might be due to the accumulation of free radicals as free radicals induce significant damage to mucosal membrane. Mucosal

defense against luminal pepsin is complicated by the mucosal action of pepsin, which, whilst it does not diffuse through the mucus, attacks the gel at the luminal surface to produce degraded glycoprotein in the gastric juice (Berstad, 1970). Two attributes of the mucus layer are its thickness and turnover rate which might be great value in protecting the mucosal layers underlying the epithelial cells (Allen, 1978). The mucosal layer is a dynamic entity in which the surface cells are continuously renewed (Bickel, 1981).

Figure 5: Histopathological sections of Stomach by Microtome on *S. rebaudiana* extract treated animals.



S. rebaudiana post treatment offered protection against the action of ethanol on gastric layers is evident by the decrease in the levels of ulcer index and increase of gastric mucosa (Borgi, 2007). The present results concur with the previous study, as extracts was significantly increased following mucosal damage. The decrease in the activity of ulcer index after *S. rebaudiana* treatment implicates its biochemical basis as an antiulcerogenic. Also, the histopathlogical observations showed that, upon *S. rebaudiana* p*ost* treatment, the mucosal epithelium had normal architecture and it had less hemorrhage as against the ethanol-induced damages in the mucosal epithelium. These observations on the cytoprotective nature of *S. rebaudiana* against ethanol-induced gastric ulcers prove its antiulcer activity.

Conclusion

In the present study, it is concluded that the gastroprotective activity seems to be related with reduction of the damage in mucosa induced by free radicals and this activity may be due to the presence of *Stevia* glycosides and other phytoconstituents like steviol, stevioside, steviolmonoside, stigmasterol, umbelliferone, quercitrin and xanthophylls. Further work is required to understand the mechanism of action which may lead to identification of potent newer anti-ulcerogenic molecules from *S. rebaudiana*.

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