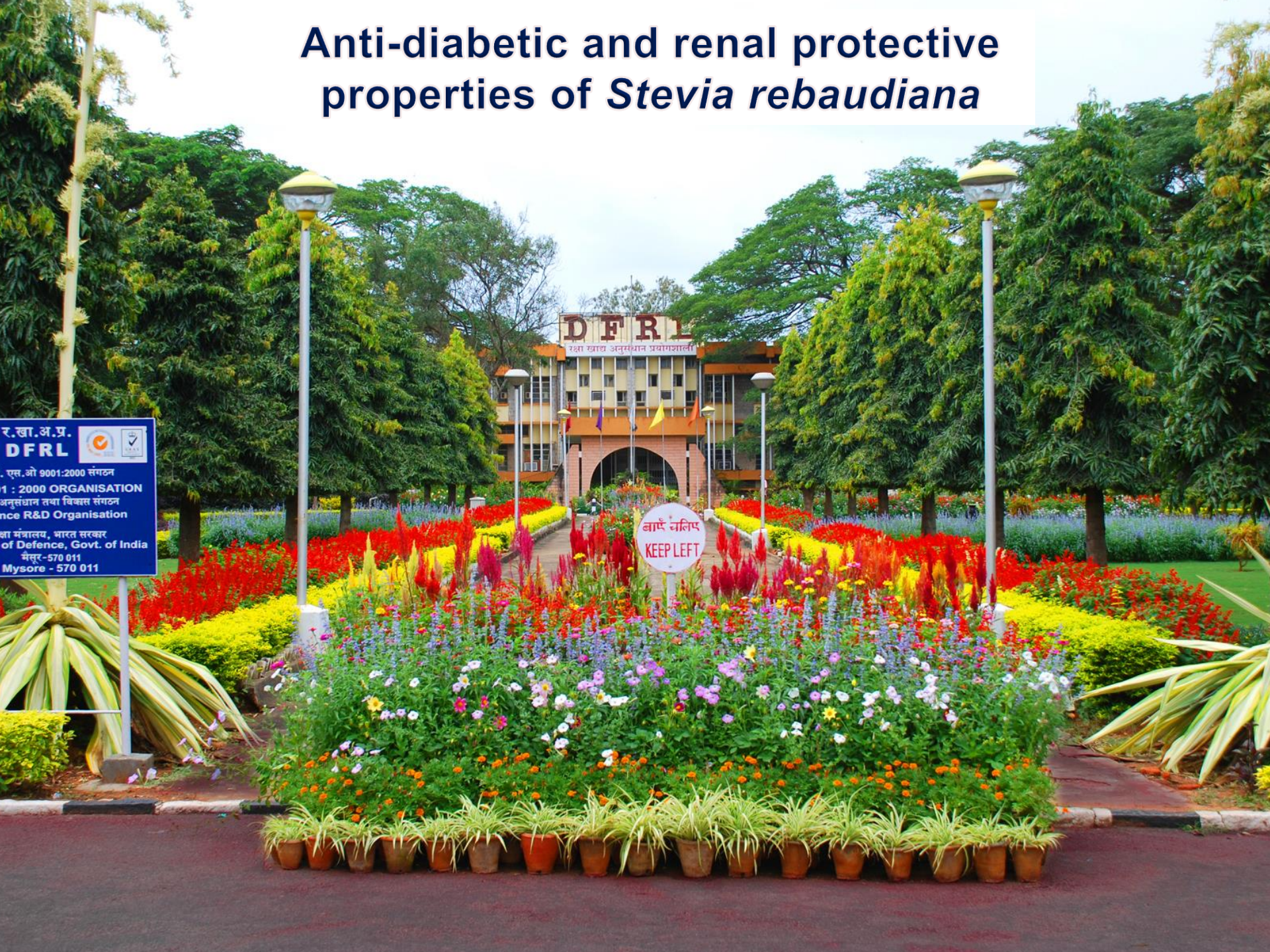


Anti-diabetic and renal protective properties of *Stevia rebaudiana*

र.खा.अ.प्र.
DFRL
एस्.ओ 9001:2000 संगठन
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अनुसंधान तथा विवस संगठन
nce R&D Organisation
क्षा मंत्रालय, भारत सरकार
of Defence, Govt. of India
मैसूर - 570 011
Mysore - 570 011



Stevia

Is an herb native to Central and South America. Also known as sugar leaf, honey leaf or sweet leaf, it's been traditionally used in Paraguay to sweeten yerba mate tea and as a medicinal herb to treat a variety of health conditions. Composed of aglycone and glycoside.



Dr Naveen S

Department of Applied Nutrition,
Defence Food Research Laboratory,
Mysore-570011. Karnataka, India.

Telephone: +91-821-2579488

Fax: +91-821-2473468

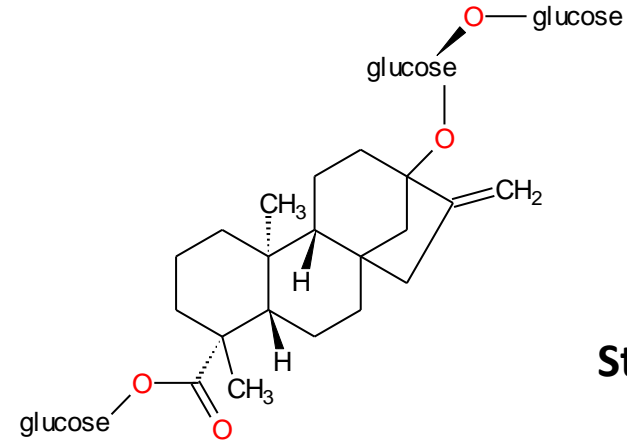
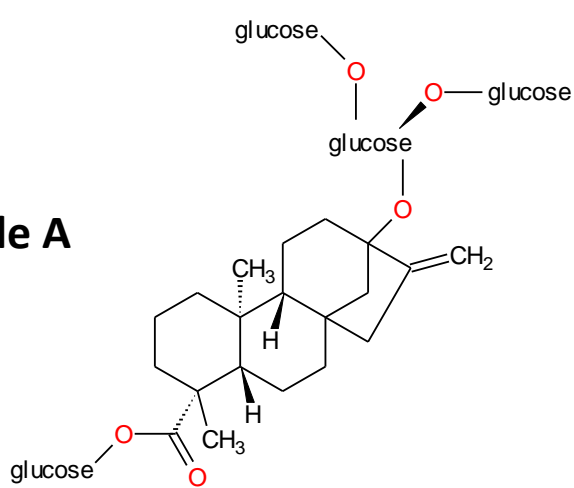
E-mail: naveen.dfri@gmail.com



- Stevia Leaves
- Liquid Extracts
- Powdered Extracts

Stevia extracts

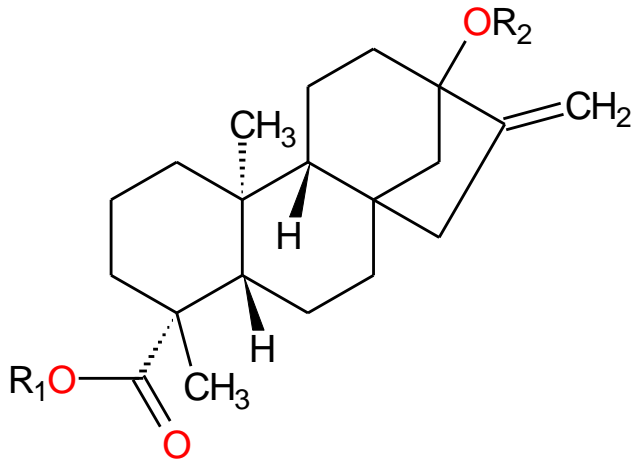
Rebaudioside A



Stevioside

- Hot-water extracts from the leaves of the Brazilian shrub *Stevia rebaudiana*
- Contains many steviol glycosides, with highest percentage of stevioside and rebaudioside A
- JECFA ADI = 4 mg/kg/day, as steviol equivalents
- Rebaudioside A preparation permitted in U.S. for food use

Stevia extracts: Metabolism



Steviol Backbone

- Glucosides not absorbed
- Steviol glycosides hydrolysed to steviol by gut microflora
 - Rate depends on number of glucose moieties attached to steviol backbone
- Steviol absorbed in large intestine, glucuronidated and excreted
 - Rats: faeces
 - Humans: urine

FDA approved Stevia

- The Food and Drug Administration has declared the herb stevia safe for use in foods and beverages, which allows Coca-Cola Co., PepsiCo Inc. and other companies to market it in a variety of products.
- Coke introduced a reduced-calorie version of Sprite, called Sprite Green, and some Odwalla juice drinks with stevia extract.
- Pepsi has SoBe Lifewater, and an orange-juice drink called Trop50, containing half the calories and sugar of orange juice.







Stevioside approval Country list

- Japan
- Korea
- China
- India
- Indonesia
- Taiwan
- South America
- Swizerland
- United States
- Canada
- Australia
- New-Zealand
- Turkey
- Thailand
- Russia



Specification Comparison

	Sucrose	Artificial Sweeteners	Steviosides	Benefit
Type	Natural	Artificial	Natural	Safe
Sweetness Level	1time	300 times	300 times	Reduce RM cost
Heat stability		90 Celsius	200Celsius	Suitable for cooking
Acid-base resistance	High	Low	High	Safe No taste changing
Safety	High	Carcinogen	Anti-carcinogenic	
Preservatives	No	No	Yes	Increase shelf-life Reduce cost of packaging
Health benefits	Diabetes Obesity	Nerve problem	blood sugar blood pressure Weight control	Value –added product
Calories	high	low	no	Low sugar food and drink



Product Application

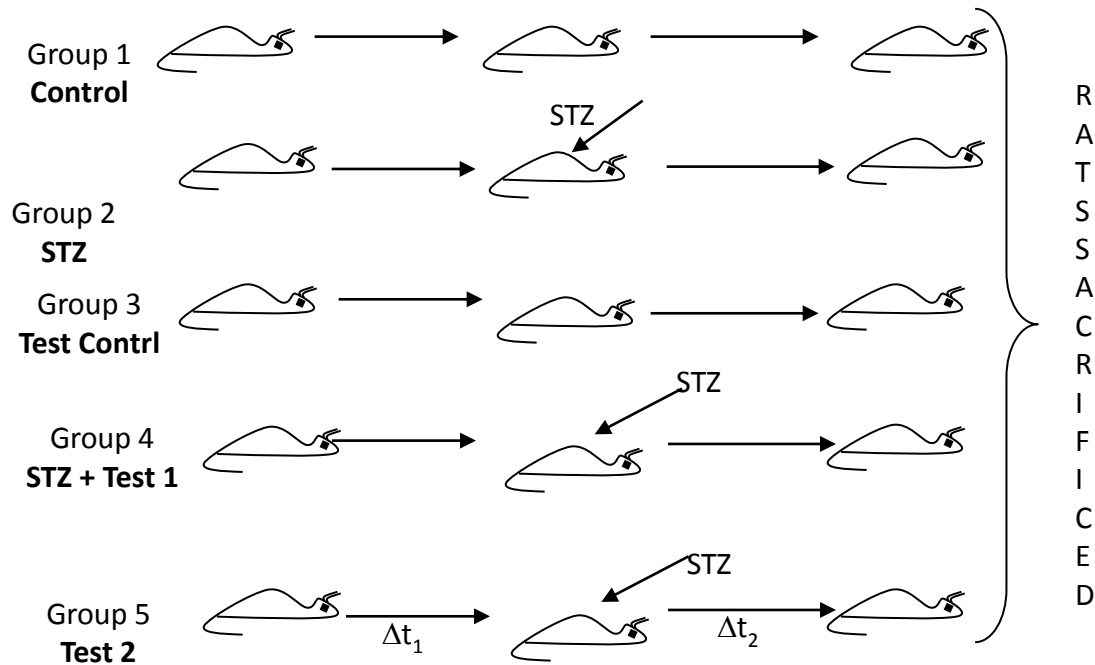
Products items	Substitute dosage for sugar	Advantages
Beverage	10-50%	<ol style="list-style-type: none">(1) Improve taste(2) Reduce high sweetness from sugar(3) It can be used for people who can not have much sugar.
Medicine	According to the requirements	<ol style="list-style-type: none">(1) Reduce the cost(2) Improve the health care function(3) Improve the taste
Cold food	10-25%	<ol style="list-style-type: none">(1) Improve the sweetness.
Caned food	20-50%	<ol style="list-style-type: none">(1) The stevia liquid is very clear and its taste is fresh.(2) Reduce the much sweetness from sugar(3) Prolong the shelf-time
Aquatic food	30-50%	<ol style="list-style-type: none">(1) It can stop the browning reaction and moldy.



Product Application

Products items	Substitute dosage for sugar	Advantages
Beer	10-40%	(1) Make the taste better. (2) It can bring much more foam; make the foam lasting and white.
Fruit wine	50%	(1) It can improve the wine's scent (2) Reduce the stickiness, improve the fresh taste.
liqueur	10-20%	(1) It can remove the hot taste from the wine

Experimental Protocol



Parameters studied:

(Liver / Heart / Kidney)

- **Lipid peroxidation:**
 - MDA
 - Hydroperoxides
 - Conjugated dienes
- **Antioxidant metabolites or molecules:**
 - GSH
 - Vitamin C
- **Antioxidant enzymes:**
 - SOD
 - GST
 - Catalase
 - GSHPx
 - GSSGR
 - G-6-PDH
- **Serum / Plasma**
 - Glucose
 - Lipid profile
 - SGOT
 - SGPT
- **Liver**
 - 1. Glycogen

- Rats were segregated randomly into 8 groups of 10 rats each.
- Groups 1 and 2 were fed with the control diet; group 3 and 4 with 4.0% stevia leaves powder incorporated diet (4.0g leaf powder in 96g dry diet);
- Group 5 and 6 with equivalent amount of polyphenols extract (through force feeding);
- Group 7 and 8 with equivalent amount of fiber extracted from 4 g of stevia leaves powder respectively for 5 weeks,
- All the rats were fed *ad libitum* with free access to water.
- Food intake and weight gain were monitored weekly.
- One week prior to sacrifice, all even groups were given a single dose of STZ (60mg/kg body weight, i.p. using a 5% solution of freshly prepared streptozotocin in 0.1 M citrate buffer pH 4.5).
- Rats were sacrificed under mild anesthesia using anesthetic ether, after which organs / tissues were quickly excised and stored in liquid nitrogen until analyses (completed immediately within a week).

RESULTS

Antioxidant activity (*in-vitro*) of Stevia leaves (n=3)

Antioxidant properties of Stevia	EC ₅₀ (μg)			
	Stevia	BHA*	BHT*	TBHQ*
1. Lipid per-oxidation inhibition property	2.6 ± 0.05	2.9 ± 0.04	2.2 ± 0.02	3.8 ± 0.02
2. Free radical scavenging property (DPPH scavenging)	10.6 ± 1.91	13.0 ± 1.72	43.2 ± 2.84	8.6 ± 0.11

*Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and Tertiary butyl hydroquinone (TBHQ).



- Antioxidant property of stevia leaves was estimated and compared with other commercial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and Tertiary butyl hydroquinone (TBHQ).
- Stevia leaves were having better antioxidant properties such as free radical scavenging and inhibition of lipid per-oxidation
- Thiobarbituric acid reaction substances, conjugated dienes and hydroperoxides; products of lipid peroxidation is a measure of oxidative stress in liver.
- The levels of malondialdehyde, conjugated dienes and hydroperoxides increased upon injection of STZ, by three times compared to untreated group.

Antioxidant property of stevia leaves
Antioxidant property of stevia leaves

Effect of Stevia on lipid peroxidation in liver in treated rats (n=8)

	TBARS (x 10 ⁻¹⁰ moles / g)	Hydroperoxides (x 10 ⁻⁴ moles / g)	Conjugated dienes (x 10 ⁻⁴ moles / g)
Control	0.06 ± 0.01 ^a	0.07 ± 0.02 ^a	0.12 ± 0.02 ^a
Streptozotocin	0.16 ± 0.03*	0.17 ± 0.03*	0.24 ± 0.06*
Stevia leaves Powder	0.08 ± 0.02 ^a	0.11 ± 0.03	0.09 ± 0.02 ^a
+ Stevia leaves Powder	0.12 ± 0.03*	0.10 ± 0.02 ^a	0.17 ± 0.04 ^a
Stevia leaves polyphenols	0.07 ± 0.01 ^a	0.09 ± 0.02	0.10 ± 0.02 ^a
+ Stevia leaves polyphenols	0.11 ± 0.02*	0.08 ± 0.01 ^a	0.14 ± 0.03 ^a
Stevia leaves fiber	0.05 ± 0.01 ^a	0.08 ± 0.02 ^a	0.15 ± 0.03 ^a
+ Stevia leaves fiber	0.17 ± 0.02*	0.15 ± 0.03*	0.25 ± 0.05*

Note: Significance is defined as $p < 0.05$. Superscript ****** indicates the value is significantly different in comparison with Control ($P < 0.05$) and superscript **'a'** indicates the value is significantly different in comparison with the treated group (STZ) at $P < 0.05$.

- Peroxidation was reduced significantly in rats prefed with stevia leaves powder and polyphenols extracted to 30-25% in liver compared to diabetic group.
- Pre-feeding stevia however did not alter the levels of antioxidants in plasma.
- STZ administration resulted in significant elevation of hepatic antioxidant enzymes SOD to 33% and catalase to 74% compared to control group, when rats were prefed with stevia leaves powder and polyphenols extracted the enzyme activity significantly reduced by 15-25% and 40-55% respectively compared to diabetic group.
- Hepatic phase I GSHPx activity was reduced by 52% on STZ treatment, which on pre-feeding with stevia leaves powder and polyphenols extracted the activity increased to 90-84% compared to diabetic group. No significant change was seen in extracted fiber fed group.

Antioxidant property of stevia leaves

Effect of stevia on Anti-diabetic action in treated rats (n=8)

	Control	Stevia	STZ	STZ + Stevia leaves powder	Stevia polyphenol	STZ + Stevia polyphenol	Stevia fiber	STZ + Stevia fiber
Weight gain (g/week)	25.8 ± 9.70	25.7 ± 14.3	6.8 ± 1.8 ^{*a}	11.7 ± 1.60 ^{*a}	21.5 ± 11.4	10.3 ± 0.95 ^{*a}	22.5 ± 9.12	6.0 ± 2.1 ^{*a}
Food intake (g/day)	9.4 ± 3.7	10.2 ± 3.00	13 ± 1.9	11 ± 3.3	9.8 ± 2.50	10.1 ± 2.3	9.1 ± 2.6	11 ± 1.1
Water intake (ml/day)	27.9 ± 16.1	20.8 ± 13.3	59.5 ± 28.4 ^{*a}	42.6 ± 23.3 ^{*a}	22.1 ± 16.1	52.6 ± 25.2 ^{*a}	25.1 ± 14.3	55.2 ± 22.1 ^{*a}
Urine volume (ml/24hrs)	39.0 ± 17.7	31.1 ± 14.7	98.4 ± 27.9 ^{*a}	57.2 ± 16.8 ^{*a}	28.3 ± 11.6	53.1 ± 11.5 ^{*a}	42.2 ± 15.1	89.9 ± 17.8 ^{*a}
Blood glucose (mg/dl)	109.2 ± 29.60	99.8 ± 22.0	356.3 ± 65.40 ^{*a}	228.2 ± 22.10 ^{*a}	101.4 ± 17.2	129.2 ± 12.50 ^{*a}	99.3 ± 10.46	342.0 ± 42.50 ^{*a}

- Treatment of animals with STZ, a known diabetogen (Marianna et al., 2006) led to polydipsia, polyphagia and polyurea.
- Food consumption increased from 120g to 300g/week, while water intake almost doubled from 20ml/day to 40ml/day. Urine output increased from 8.0ml to 15ml/day.
- The body weight which was 306g on the day of injection of STZ was 240g at the weekend i.e, a loss of 30% in one week.
- Pre-feeding the rats with stevia leaves powder and polyphenols extracted before injecting STZ prevented these changes significantly; food consumption was brought down by 20-16%, water consumption by 55-50%, urine output by 55-47% and loss in body weight was limited to 5-10g/week by stevia leaves powder and polyphenols extracted.
- The diabetic rats showed a significant increase in the blood glucose level when compared to the normal rats (P<0.05).
- Stevia leaves powder and its polyphenol extract reduced the elevated levels of blood glucose by 50-30% compared to the untreated diabetic control (P<0.05). but there was no reduction in blood glucose level in stevia fiber fed rats.
- In the STZ group, the serum insulin level was lower than that of the normal group (P<0.05).

Anti-diabetic action of Stevia leaves
Anti-diabetic action of Stevia leaves

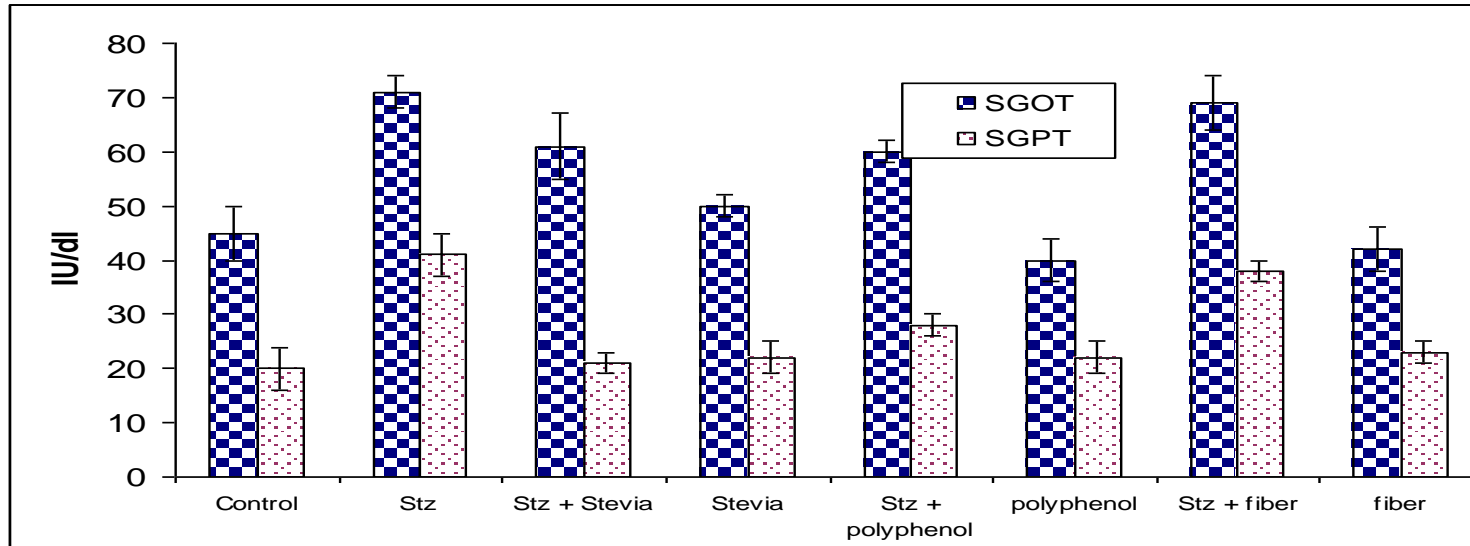
Effect of stevia on kidney functions in treated rats (n=8)

	Control	Stevia	STZ	+ Stevia leaves powder	Stevia polyphenol	+ Stevia polyphenol	Stevia fiber	+ Stevia fiber
Plasma Creatine	0.47 ± 0.11	0.40 ± 0.14	0.57 ± 0.02	0.53 ± 0.12	0.50 ± 0.22	0.45 ± 0.09	0.48 ± 0.08	0.60 ± 0.10
Urinary Creatine	0.071 ± 0.003	0.082 ± 0.014	0.058 ± 0.002* ^a	0.062 ± 0.002* ^a	0.080 ± 0.01	0.059 ± 0.008* ^a	0.069 ± 0.01	0.059 ± 0.004* ^a
GFR (ml/min/kg body wt)	5.55 ± 0.24 ^a	6.42 ± 0.32*	4.56 ± 0.77 ^a	4.89 ± 0.18 ^a	6.01 ± 0.12*	4.95 ± 0.06 ^a	5.49 ± 0.11 ^a	4.44 ± 0.20 ^a

- A slight reduction, though not significantly; in the kidney weight was observed in stevia leaves powder and polyphenols extracted groups, which increased on STZ treatment.
- Also there was an increase in GFR in STZ treated rats and was reduced on pre-feeding with stevia leaves powder and polyphenols extracted.
- The data on the effect of stevia on kidney antioxidant enzymes viz. G-6-PDH, and detoxifying enzyme γ -glutamyl transpeptidase showed administration of STZ resulted in decreased G-6-PDH and increased γ -glutamyl transpeptidase activity.
- The STZ-induced elevation in renal G-6-PDH was brought down by stevia leaves powder but both the enzyme activities were brought down by the polyphenols extracted.
- No significant change was seen in extracted fiber fed group.

Renal protective activity/capacity Stevia leaves
Renal protective activity/capacity Stevia leaves

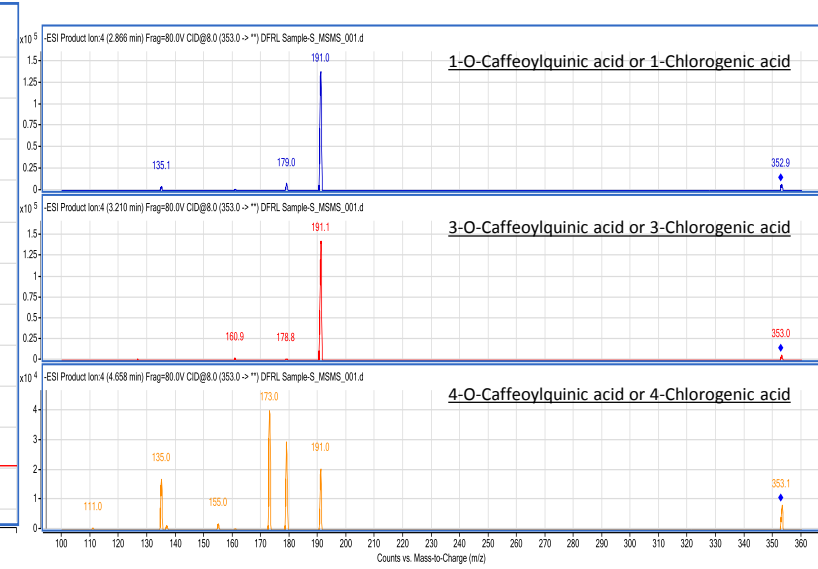
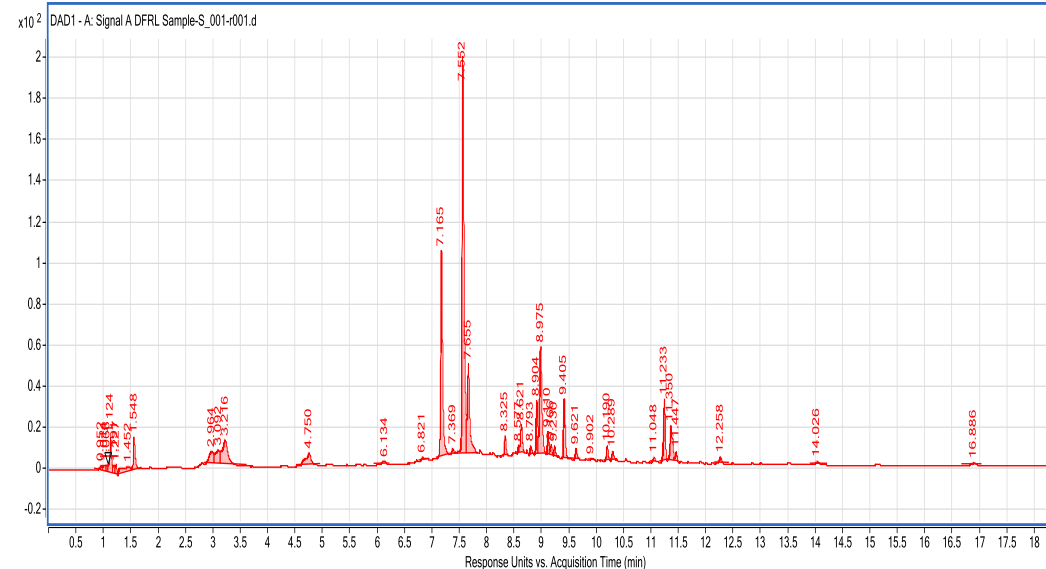
Effect of Stevia leaves on hepatic marker enzymes of STZ treated rats (n=8)



- Normal values of ALT and AST in serum for rats is 46-80 and 18-30IU/dl respectively (Kalyanasundarm, 1985).
- Serum ALT and AST levels were significantly increased in STZ treated animals by 89 and 42% compared to control group (Fig. 4).
- Pre-feeding of stevia leaves powder and extracted polyphenols to diabetic animals reduced the activity to 45-38 and 13-6% compared to diabetic group respectively.
- No significant change was seen in extracted fiber fed group.

Hepatoprotective action of Stevia leaves

Hepatoprotective action of Stevia leaves



- The identification of major polyphenols was based on comparison of their retention times with those of reference compounds and their elution order on reversed-phase C18 columns.
- Peak assignment was confirmed by mass spectrometry.
- Major compounds identified are dicaffeoylquinic acid, chlorogenic acid, Quercetin 3-O-xyloside, Apigenin-7-O-glucoside, 3,4-Dimethoxycinnamic acid, Luteolin 7-O-rutinoside, Caffeic acid etc
- Stevia was found to oppose the hyperglycemic actions in the diabetic treated rats.
- Previous studies had also noted the importance of hypoglycemic components of Stevia is because of Rebaudioside A and stevioside that are concentrated in leaves.
- In our study none of the compounds identified in the polyphenol extract was Rebaudioside A or stevioside, since the mass was not matching with compounds detected.
- This explains the involvement of polyphenolic compounds in preventing diabetic and its complications caused by streptozotocin

Phenolic compounds identification in Stevia leaves

CONCLUSIONS

- All these results are quite interesting and very encouraging.
- One single plant could have multifaceted benefits.
- However, the study brings forth many questions which need further thorough investigation, like the mechanism of action of stevia leaves powder inactivating the STZ in the blood itself, or does it prevent its transport across into pancreatic and hepatic cells? Whether the extract simply mops off the reactive oxygen species or is it a chain breaking antioxidant? Whether the prevention of the liver damage seen with stevia leaves is the result of its antioxidant property or does it induce the activity of any other liver enzymes? The plant seems to be worth investigating further and could be an excellent source for nutraceuticals.
- In conclusion, polyphenols present in stevia leaves are mainly responsible for its multi-beneficial properties.

Thank you for attention

