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Research Article

Evaluation of Anti diabetic activity of Ethanolic Extract of Beet Root (EEBT- *Beta vulgaris)* against Streptozocin induced diabetic Rats.

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ABSTRACT

Diabetes mellitus is a heterogeneous group of diseases characterized by chronic elevation of glucose in the blood. It arises because the body is unable to produce enough insulin for its own needs, either because of impaired insulin secretion, impaired insulin action, or both. Chronic exposure to high blood glucose is a leading cause of renal failure, visual loss and a range of other types of tissue damage. Diabetes also predisposes to arterial disease, not least because it is often accompanied by hypertension, lipid disorders and obesity. Many cases of diabetes and almost all of its unwanted long-term consequences are potentially avoidable, but this will require intervention at a societal as well as at a medical level. The three classic symptoms of diabetes are thirst, polyuria and weight loss. As glucose is lost in the urine it draws fluid and other small molecules with it, causing excessive urination, which in turn causes dehydration and thirst. Weight is lost because of rapid breakdown of fat and protein reserves to compensate for the loss of glucose and metabolic inefficiency due to lack of insulin action. These symptoms may be less prominent in older people with type 2-diabetes, who may present with symptoms less directly related to diabetes, or with complications of diabetes ranging from infections to heart disease, or simply as the result of a screening blood test. The main objective of the present work is to screen the bioactive molecules and evaluate the anti diabetic activity of ethanolic extracts of **beet root *(Beta vulgaris).*** The anti diabetic activity was carried out in streptozocin induced diabetic rats. The present experimental data displayed that in STZ induced diabetic rats, the blood glucose levels were in the range of 278–280 mg/dL, which were considered as severe diabetes. In the glibenclamide (5 mg/kg) and EEBT (400 mg/kg) treated groups, the peak values of blood sugar significantly decreased from 282.2 mg/ dL to 116.6 mg/dL and from 280.6 mg/dL to 118.2 mg/dL on the 21st day, respectively. Hence, from this experimental data showed that the EEBT significantly decrease the blood glucose level in diabetic rats but values did not return to those of normal controls. Therefore, EEBT possesses significant ($P < 0.01$) ant diabetic activity, when compared with diabetic control. There was significant reduction in blood sugar level, serum lipids, urinary sugar and ketone bodies (in 21 days) in STZ diabetic animals.

Key Words: Diabetes mellitus, renal failure, polyuria, STZ etc.

INTRODUCTION [1-5]

Beta vulgaris (beet) is a plant in the Amaranthaceae family (which is now included in Betoideae subfamily). It has numerous cultivated varieties, the most well known of which is the root vegetable known as the beetroot or garden beet. Other cultivated varieties include the leaf vegetable chard; the sugar beet, used to produce table sugar; and mangelwurzel, which is a fodder crop. Three subspecies are typically recognised. All cultivated varieties fall into the subspecies Beta vulgaris subsp. vulgaris. Beta vulgaris subsp. maritima, commonly known as the sea beet, is the wild ancestor of these and is found throughout the Mediterranean, the Atlantic coast of Europe, the Near East, and India.

The taxonomy of the various wild and cultivated races of beets has a long and complicated history. Mansfeld's Encyclopedia of Agricultural and Horticultural Crops following Letschert's 1993 treatment of Beta, section Beta recognizes the following taxa [30] for cultivated varieties, which are grown for their taproots, leaves, or swollen midribs: B. v. ssp. vulgaris convar. cicla (leaf beet or chard) - The leaf beet group has a long history dating to the second millennium BC. The first cultivated forms were believed to have been domesticated in the Mediterranean, but were introduced to the Middle East,

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India, and finally China by 850 AD. These were used as medicinal plants in Ancient Greece and Medieval Europe. Their popularity declined in Europe following the introduction of spinach. B. v. ssp. v. convar. cicla. var. cicla (spinach beet) - This variety is widely cultivated for its leaves, which are usually cooked like spinach. It can be found in many grocery stores around the world [6].

Figure 1: Yellow-stemmed chard (with purple-leaved kale).

Figure 2: Beet root plant

Possible health benefits of consuming beetroot [7]

Consuming fruits and vegetables of all kinds has long been associated with a reduced risk of many lifestyle-related health conditions. Many studies have suggested that increasing consumption of plant foods like beetroot decreases the risk of obesity and overall mortality, diabetes, heart disease and promotes a healthy complexion and hair, increased energy, overall lower weight. Heart health and blood pressure: A 2008 study published in Hypertension examined the effects of ingesting 500 mls of beetroot juice in healthy volunteers and found that blood pressure was significantly lowered after ingestion. Researchers hypothesized this was likely due to the high nitrate levels contained in beet juice and that the high nitrate vegetables could prove to be a low cost and effective way to treat cardiovascular conditions and blood pressure. Another study conducted in 2010 found similar results that drinking beetroot juice lowered blood pressure considerably on a dose-dependent basis.

Dementia: Researchers at Wake Forest University have found that drinking juice from beetroot can improve oxygenation to the brain, slowing the progression of dementia in older adults. According to Daniel Kim-Shapiro, director of Wake Forest's Translational Science Center, blood flow to certain areas of the brain decrease with age and leads to a decline in cognition and possible dementia. Consuming beetroot juice as part of a high nitrate diet can improve the blood flow and oxygenation to these areas that are lacking.

Diabetes: Beets contain an antioxidant known as alpha-lipoic acid, which has been shown to lower glucose levels, increase insulin sensitivity and prevent oxidative stress-induced changes in patients with diabetes. Studies on alpha-lipoic acid have also shown decreases in peripheral neuropathy and/or autonomic neuropathy in diabetics. However, a meta-analysis suggests that the benefits of alpha-lipoic acid for symptomatic peripheral neuropathy may be restricted to intravenous consumption of the acid; the authors conclude that "it is unclear if the significant improvements seen after 3-5 weeks of oral administration at a dosage of >600 mg/day are clinically relevant."6

Digestion and regularity: Because of its high fiber content, beetroot helps to prevent constipation and promote regularity for a healthy digestive tract.

Inflammation: Choline is a very important and versatile nutrient in beetroot that helps with sleep, muscle movement, learning and memory. Choline also helps to maintain the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat and reduces chronic inflammation.

Exercise and athletic performance: Beetroot juice supplementation has been shown to improve muscle oxygenation during exercise, suggesting that increased dietary nitrate intake has the potential to enhance exercise tolerance during long-term endurance exercise. Quality of life for those with cardiovascular, respiratory, or metabolic diseases, who find the activities of daily living physically difficult because of lack of oxygenation, could be improved. Beetroot juice improved performance

by 2.8% (11 seconds) in a 4-km bicycle time trial and by 2.7% (45 seconds) in 16.1-km time trial.

Potential health risks of consuming beetroot

If improperly stored, nitrate-containing vegetable juice may accumulate bacteria that convert nitrate to nitrite and contaminate the juice. High levels of nitrite can be potentially harmful if consumed. A high-nitrate diet may interact with certain medications such as organic nitrate (nitroglycerine) or nitrite drugs used for angina, sildenafil citrate, tadalafil, and vardenafil.2 Drinking beetroot juice may cause red urine or stool. It is the total diet or overall eating pattern that is most important in disease prevention and achieving good health. It is better to eat a diet with variety than to concentrate on individual foods as the key to good health.

MATERIALS AND METHOD

Drugs and chemicals used: Glibenclamide, streptozotocin (STZ), and sodium citrate buffer were used in this study. Other chemicals used for extraction purpose and phytochemical tests were of laboratory grade.

Experimental animals: White male albino Wister rats weighing about 200-250gm was used, they were obtained from the animal house of C. L. Baid metha College of Pharmacy, chennai. They were kept under observation for about 7 days before onset of experiment to exclude any intercurrent infection, had free access to normal diet and water. The experimental protocol was approved by **IAEC** (Institutional Animal Ethics Committee) of **CPCSEA: IAEC/XXIX/12/2016.**

Methodology [8]: Weigh 20 g of beet root paste (root can be mashed or grined to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separating funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating and obtained ethanolic extract of beet root **(EEBT) of *Beta vulgaris*.**

Phytochemical screening [9-11]: Preliminary Phytochemical screening of **EEBT** had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc.

Evaluation of acute oral toxicity [12]: In the present study the acute oral toxicity of the EEBT was performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b. w. Animals were observed individually after dosing at least once during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. No animal died. Therefore, the LD_{50} is greater than 2000 mg/kg. An investigation with 1/20th, 1/10th, and 1/5th of 2000 mg/ kg, i.e. 100, 200, and 400 mg was done in pre-screening. Only 400 mg/kg was found to be effective against diabetes, hence this dose was used in final screening. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of **CPCSEA: IAEC/XXIX/12/2015.**

Evaluation of Anti diabetic Activity [13–15]: After fasting, DM was induced by intraperitoneal injection of STZ dissolved in 0.1 M cold sodium citrate buffer (pH 4.4) at a dose of 30 mg/kg b. w. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. After 72 h, STZ-treated animals were considered as diabetic when the fasting plasma levels were observed above 200 mg/dL with glycosuria. The experiment was carried out with four groups of animals and five rats were used in each of the four groups which were given as below:

A. Group I: Normal control (vehicle).
B. Group II: Diabetic control (vehicle).
C. Group III: Diabetic rats treated with EEBT (400 mg/kg p. o.)
D. Group IV: Diabetic rats treated with glibenclamide (5 mg/kg p. o.)

Vehicle, EEBT, and glibenclamide were administered once daily for 21 days from the day of induction. Blood was drawn from tip of the tail, and blood glucose level was estimated on 0, 7th, 14th, and 21st day of experiment with the help of glucometer using strip method. On 21st day, blood sample was collected by retro orbital puncture for measuring serum cholesterol and TG level using auto analyser. Fresh urine was collected for the determination glucose and ketone bodies, by using keto-diastix strips on 0 and 21st day of the experiment.

Statistical analysis

All results were expressed as mean ± SEM. The data were analyzed using analysis of variance (ANOVA), and the group means were compared by Dunnett's test. Values

were considered statistically significant with $P < 0.05$. Graph Pad Instat was used for the analysis of data.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Preliminary Phytochemical screening of **EEBT** had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc.

Acute oral toxicity study:

(i) Acute oral toxicity studies were performed according to the OECD guideline 423 method.
(ii) This method has been designed to evaluate the substance at the fixed doses and provide information both for hazard assessment and substance to be ranked for hazard classification purposes.
(iii) The **EEBT** was administered initially at a dose of 2000 mg/kg b.w and 1% CMC (p.o) and observed 14 days mortality due to acute toxicity.
(iv) Careful observation were made at least thrice a day for the effect on CNS, ANS, motor activity, salivation and other general signs of toxicity were also observed and recorded.
(v) Since no sign of toxicity observed at 2000 mg/kg b.w. to the group of animals, the **LD_{50}** value of the **EEBT** expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD50 < 2500 mg/kg).
(vi) From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there **LD_{50}** cut of values > 2000 mg/kg b. w.

Table 1: for the dose selection by acute toxicity class method (OECD) guide lines 423 of EEBT

Sl. No.	Treatment group	Dose mg/kg	Sign of toxicity	Onset of toxicity	Duration
1	**EEBT**	**200**	**No**	**No**	**14 days**

Blood glucose level: In STZ induced diabetic rats, the blood glucose levels were in the range of 278–280 mg/dL, which were considered as severe diabetes. In the glibenclamide (5 mg/kg) and EEBT (400 mg/kg) treated groups, the peak values of blood sugar significantly decreased from 282.2 mg/ dL to 116.6 mg/dL and from 280.6 mg/dL to 118.2 mg/dL on the 21st day, respectively. Hence, in this experimental data displayed that the EEBT significantly decrease the blood glucose level in diabetic rats but values did not return to those of normal controls. Therefore, EEBT possesses significant ($P < 0.01$) ant diabetic activity, when compared with diabetic control. There was significant reduction in blood sugar level (in 21 days) in STZ diabetic animals.

Table 2: Effect of EEBT on blood glucose level in STZ induced diabetic rats

Groups	**Blood glucose (mg/dL) in day**			
	0	**7**	**14**	**21**
I	92.2 ± 3.55	94 ± 2.3	94.8 ± 3.05	95.2 ± 2.37
II	278 ± 6.20	281.5 ± 6.01	282.6 ± 5.82	283.4 ± 5.24
III	280.6 ± 5.14^{ns}	257.6 ± 4.96**	190.4 ± 5.25**	118.2 ± 2.28**
IV	282.2 ± 6.88 ^{ns}	247.8 ± 5.76**	178.4 ± 6.49**	116.6 ± 3.19**

Values are mean ± SEM; n = 5 in each group except in diabetic control group where n = 4 because one animal died on the 8th day. Ns P > 0.05 (non-significant), **P < 0.01 (highly significant) when compared to diabetic control rats; EEBT or glibenclamide was administered daily for 21 days. For glucose estimation, blood was collected just before the drug administration on the 0 day and 1 h after the drug administration on the 7th, 14th day and 21st day.

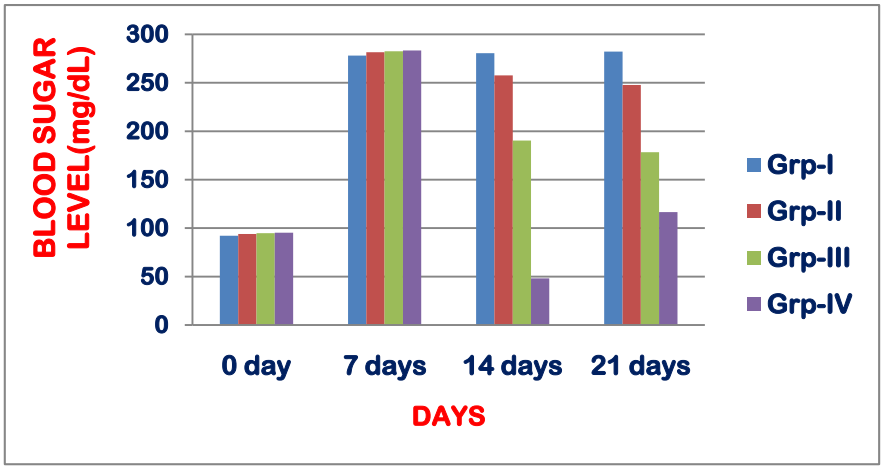

Figure 3: The effect of EEBT and glibenclamide on blood glucose level in STZ induced diabetic rats at various days (on 0 day, 7th day, 14th day, and 21st day).

Serum lipid profile: The serum profile lipids also determined by analysis of serum sample withdrawn from the all groups. The untreated diabetic rats' serum levels of cholesterol and TG were significantly increased which was significantly declined with administration of EEBT. The results obtained from the glibenclamied on serum TG and cholesterol in the diabetic rats was comparable to those of the herbal EEBT. Total cholesterol and TG were significantly increased in diabetic group in comparison to control group. Administration of EEBT for 21 days potentially decreased the serum levels of cholesterol and TG in comparison to diabetic control rats.

Table 3: Effect of EEBT on serum lipid profile in STZ-induced diabetic rats

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)
I	80.12 ± 2.59	68.3 ±4.15
II	129 ± 9.39	119.5 ± 4.94
III	101.3 ± 4.03*	93.9 ± 2.94*
IV	86.18± 2.11**	82.12 ± 5.43**

Values are mean ± SEM; n = 5 in each group except in diabetic control group where n = 4 because one animal died on the 8th day. *P < 0.05 (significant),**P < 0.01 (highly significant) when compared to diabetic control rats.

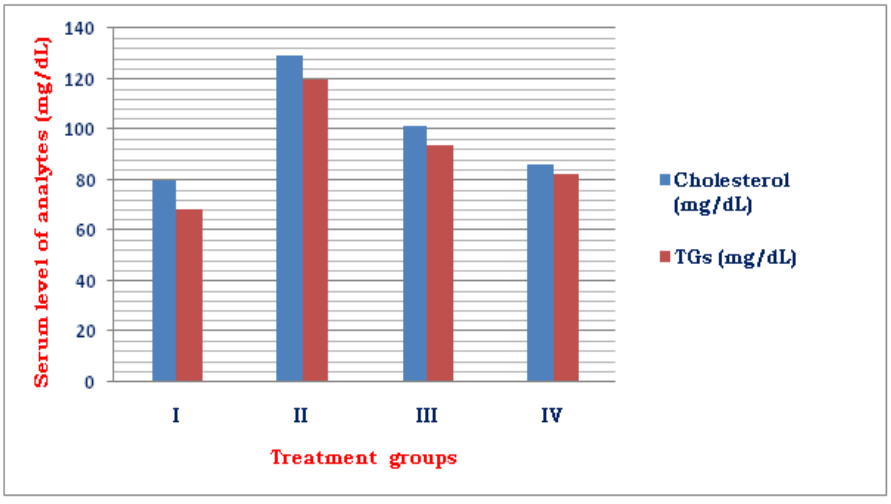

Figure 4: The effect of EEBT and glibenclamide on blood lipids level in STZ induced diabetic

Determination of urinary glucose and Ketone bodies: Urine analysis on day 0 displayed the presence of glucose (+++) and ketone (trace) ina all groups, except normal control and on 21st day glucose and ketone traces were absent in EEBT and glibenclamide treated groups where as urinary glucose and ketone bodies were present in diabetic control group.

Table 3: Effect of EEBT on urinary glucose and ketone bodies in STZ-induced diabetic rats

Groups	0 day		21st day	
	Glucose	Ketone bodies	Glucose	Ketone bodies
I	–	–	–	–
II	+++	Trace	+++	Trace
III	+++	Trace	–	–
IV	+++	Trace	–	–

- = absence of glucose, +++ = 1.1 g/dL, - = absence of ketone, Trace = 4.98 mg/dL

CONCLUSION

From the present experimental data, here I concluded that the EEBT possessed potential anti diabetic activity against streptozocin induced rats which was proved by assessment of bioanalytes from serum and urine sample.

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