

# Evaluation of Antidiabetic and Antioxidant Effects of Seabuckthorn (*Hippophae rhamnoides L.*) in Streptozotocin-Nicotinamide Induced Diabetic Rats

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**Abstract:** Seabuckthorn (*Hippophae rhamnoides L.*) is a branched deciduous shrub native to Europe and Asia. The plant has been used extensively in oriental traditional system of medicine for treatment of different diseases. Seabuckthorn is found to have significant antioxidative, antimicrobial, immunomodulatory, cytoprotective, hepatoprotective and tissue regenerative properties. The present study was undertaken to evaluate the antidiabetic and antioxidant effect of seabuckthorn (*Hippophae rhamnoides L.*) in streptozotocin-nicotinamide induced type-2 diabetic rats. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg), 15 minutes after the i.p. administration of 120mg/kg nicotinamide. Seabuckthorn was administered orally to streptozotocin (STZ) diabetic rats. Blood glucose, tissue glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in pancreas were estimated following the established procedures. Biochemical observations were supplemented with histological examination of pancreatic tissue. The increase of blood glucose, TBARS level with reduction in GSH content were the salient features observed in STZ- diabetic rats. Sea buckthorn produced a significant ( $p < 0.05$ ) reduction in blood glucose levels and TBARS levels in the STZ- diabetic rats. GSH, reduced significantly ( $p < 0.05$ ) in diabetic rats, was brought back to near normal levels by co-administration of sea buckthorn. Degenerative changes of pancreatic beta cells in STZ- diabetic rats were minimized to near normal morphology by administration of sea buckthorn as evident by histopathological examination. The results of the study indicate the role of oxidative stress in the induction of diabetes and suggest a protective effect of sea buckthorn in this animal model.

**Keywords:** Antioxidant, diabetes, oxidative stress, seabuckthorn, streptozotocin.

## 1. INTRODUCTION

India continues to be the 'Diabetic Capital' of the world with 50.8 million diabetics. The pandemic spread of type 2 diabetes and identification of new therapeutic avenues in the treatment of all pathological aspects of this disorder remain a major challenge for current biomedical research. Plant based herbal drugs are emerging as the primary components of holistic approaches to the diabetes management. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicines to natural products that may be better treatment than the currently used drugs [1].

Streptozotocin is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic  $\beta$ -cells. STZ-induced diabetes mellitus is associated with the generation of reactive species causing oxidative damage [2]. Diabetics and experimental models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes *de novo* free radicals generation [3]. Over the past few decades there has been increasing scientific and

public interest in so called antioxidant hypothesis. Therefore, in addition to control of blood glucose levels, control of oxidative stress offers another avenue for the treatment of the disease. Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of  $\beta$  cells and protect pancreatic islets against the cytotoxic effects of STZ [4].

Seabuckthorn (*Hippophae rhamnoides L., Elaegnacea*) is a thorny deciduous shrub native to several countries of Europe and Asia. The distribution ranges from Himalaya regions of India, Nepal, Pakistan, Afghanistan, Britain, Germany, Finland and France. All parts of this plant are considered rich source of a large number of bioactive substances with high medicinal and nutritional properties. Seabuckthorn (SBT) seed oils, berries, leaves and bark are well known for their medicinal properties and have been suggested to be due to high contents of antioxidative substances present in this plant. It has been extensively investigated for its antioxidative, antimicrobial, immunomodulatory, cytoprotective, antiatherogenic, hepatoprotective and tissue regenerative properties [5-8]. Furthermore, earlier studies have reported that SBT berries are the rich source of vitamins A, C, E, and K, flavonoids, carotenoids, organic acids and oils [9]. Many medicinal effects of SBT berries, such as, improving functions of digestive system and blood circulation have also been reported [10]. Further, it is

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reported to be well tolerated and without any untoward reaction or side effects in toxicological studies [11]. It is interesting to note that SBT has recently been reported to possess hypoglycemic activity [12]. In view of all the above reports, we chose to evaluate SBT for possible beneficial effect on STZ-induced hyperglycemia and oxidative stress.

## 2. METHODOLOGY

### 2.1. Animals

Male albino Wistar rats (150-200 g) were used for the study. They were housed in polypropylene cages under standard laboratory conditions (12 h light/12 h dark, 21±2° C). The animals were fed on a standard pellet diet (Lipton rat feed Ltd., Pune) and water *ad libitum*. The experimental study was approved by the Institutional Animal Ethical Committee of Jamia Hamdard.

### 2.2. Drugs and Chemicals

Commercially available preparation of Seabuckthorn (*Hippophae rhamnoides L.*, fruit pulp was procured from Panacea International, New Delhi, India. Streptozotocin was procured from Sigma Chemicals Co., St. Louis, MO, USA. All other biochemicals and chemicals used for the experiment were of analytical grade.

### 2.3. Induction of Diabetes

The animal model of type-2 diabetes mellitus (NIDDM) was induced [13] in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ, 15 min after the i.p. administration of 120 mg/kg nicotinamide. Hyperglycaemia was confirmed by the elevated blood-glucose levels determined at 72 h.

### 2.4. Experimental Design

The rats were divided into six groups comprising of six animals in each group as follows:-

- Group I: Normal control rats, received normal saline (1 ml/kg)
- Group II: Diabetic control rats, received STZ in single dose i.p. (60 mg/kg, 15 min after the i.p. administration of 120 mg/kg nicotinamide)
- Group III: SBT treated rats received (SBT 2 ml/kg, p.o.)
- Group IV: Diabetic treated rats received (SBT 1 ml/kg, p.o.)
- Group V: Diabetic treated rats received (SBT 2 ml/kg, p.o.)
- Group VI: Diabetic treated rats received Gliclazide (25 mg/kg, p.o.)

Drug treatment was given for duration of 3 weeks.

SBT was given for a period of 3 weeks to group III, IV & V animals. On the last day of the experiment blood samples were collected for biochemical estimations.

### 2.5. Determination of Blood Glucose

Blood glucose level was estimated by glucose oxidase [14] method using a commercial diagnostic kit from Span Diagnostic Ltd., Surat, India.

### 2.6. Determination of HbA1c Level

Glycosylated haemoglobin (HbA1c) level was estimated by [15] method using a commercial diagnostic kit from Monozyne India Limited, Secunderabad, India.

### 2.7. Determination of Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) was measured according to the method of Pari and Saravana [16]. Glucose solution (2 g/kg) was given to overnight fasted rats. Blood samples were taken at 0, 15, 30, 60 and 120 min. after glucose administration. All the blood samples were collected for glucose estimation.

### 2.8. Determination of Lipid Peroxidation

Lipid peroxidation (LPO) was measured by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids [17]. Tissues were homogenized in chilled phosphate buffer (0.1M, pH 7.4) that contained KCl (1.17%w/v), using motor driven Teflon pestle. Aliquot of 1ml of the suspension medium was taken from the supernatant obtained after the centrifugation of tissue homogenate (10% w/v) at 10,500 × g. About 0.5ml of 30% trichloroacetic acid (TCA) followed by 0.5ml of 0.8% TBA was then added to it. The tubes were kept in shaking water bath for 30 min. at 80 °C. After 30 min. of incubation tubes were taken out and kept in ice cold water for 10 min. These were then centrifuged at 800× g for 15 min. The absorbance of supernatant was read at 540nm at room temperature against appropriate blank. The concentration of MDA was measured from the standard calibration curve prepared by using tetraethoxypropane (TEP). Protein was estimated by the method of Lowry *et al.* (1951) [18]. Lipid peroxidation was expressed as n moles of MDA per mg of protein.

### 2.9. Determination of GSH

GSH content was estimated by method of Sedlak and Lindsay, 1968 [19]. The tissues were homogenized in 0.02M EDTA. Aliquots of 5ml of the homogenates were mixed in the test tube with 4ml of cold distilled water and add 1ml of 50% TCA. The tubes were shaken for 10 min using vortex mixer and then centrifuged at 1200 × g for 15min. Following centrifugation 2ml of supernatant was mixed with 4ml of 0.4M Tris-buffer (pH8.9). The whole solution was mixed and 0.1 ml of 0.01 M DTNB {5, 5'- dithiobis (2-nitrobenzoic acid)} was added to it. The absorbance was read within 5min of addition of DTNB at 412nm using UV-spectrophotometer (Shimadzu, UV-1601, Japan) against a reagent blank with no homogenate.

### 2.10. Histopathological Examination of Pancreas

The pancreas was isolated immediately after sacrificing the animal and washed with ice cold saline. It was then fixed in 10% neutral buffered formalin solution. Sections of 3-5µm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination.

### 2.11. Statistical Analysis

Data were expressed as the mean ± standard error (S.E.) of the means. For a statistical analysis of the data, group means were compared by one-way ANOVA with *post-hoc*

**Table 1. Effect of SBT and Gliclazide on Blood Glucose and Glycosylated Haemoglobin Levels in STZ Diabetic Rats**

Group	Treatment	Glucose in mg/dl (Day 0)	Glucose in mg/dl (Day 21)	Glycosylated Haemoglobin (%)
1	Normal saline (1 ml/kg, p.o.)	69.83±3.46	67.83±6.72	5.70±0.6
2	STZ (60 mg/kg, i.p.) + NIC (120 mg/kg, i.p.)	212.5±28.87**	226.08±18.33	12.18±0.32
3	Sea buckthorn fruit pulp (2ml/kg, p.o.)	66.58±7.99 <sup>ns</sup>	71.83±7.42	5.67±0.15
4	STZ ±NIC+SBT (1 ml/kg, p.o.)	226.24±29.57**	103.70±15.64** <sup>ns</sup>	9.30±0.12
5	STZ±NIC+SBT (2 ml/kg, p.o.)	230.43±35.27**	93.75±12.07** <sup>ns</sup>	6.95±0.7** <sup>ns</sup>
6	STZ + NIC + Glic (25mg/kg, p.o.)	198.13±27.8**	79.122±9.57** <sup>ns</sup>	5.3±0.7** <sup>ns</sup>

Data are expressed in Mean ± SEM, n=6, for Day 0 comparison were made vs Group 1(positive control) and for Day 21 vs Group 2 (Diabetic control) and Group 6 (Gliclazide standard) - \*\* and ns statistically significance at p<0.01 and non significance respectively.

analysis.  $P < 0.05$  was considered to be statistically significant.

### 3. RESULTS

#### 3.1. Effects of SBT on Hyperglycemia in STZ –Induced Diabetic Rats

Table 1 shows the effect of SBT on the blood glucose level. Significant ( $P<0.001$ ) increase in blood glucose level was observed in STZ induced –diabetic rats when compared to normal control rats. Oral administration of SBT at two doses (1 and 2 ml/kg), for 3 weeks reduced the blood glucose level significantly ( $P<0.001$ ) in a dose dependent manner. On its own SBT did not register any significant change in blood glucose control values when compared to normal control rats. Gliclazide has its expected glucose lowering effect in diabetic animals.

#### 3.2. Effect of SBT on HbA1c in STZ –Induced Diabetic Rats

Table 1 shows the effect of SBT on HbA1c level. Significant increase in HbA1c level was observed in STZ diabetic rats when compared to normal control rats. Oral administration of SBT at two doses (1 and 2 ml/kg), for 3 weeks decreased the HbA1c level significantly ( $P<0.001$ ) in a dose dependent manner. There was no significant change in HbA1c level of SBT treated rats when compared to normal control rats.

#### 3.3. Effect of SBT on OGTT in STZ –Induced Diabetic Rats

Table 2 shows the blood glucose levels of normal control, diabetic control and SBT treated diabetic rats after oral administration of glucose (2 g/kg). In STZ diabetic rats, the peak increase in blood glucose level was observed after 1hr. The blood glucose level remained high over next 1hr. SBT significantly reduced the blood glucose level in glucose- loaded rats at 30, 60, 90 and 120 min.

#### 3.4. Effect of SBT on LPO in STZ –Induced Diabetic Rats

Table 3 demonstrates the level of malondialdehyde (MDA), a secondary product of lipid peroxidation in the

pancreatic tissue homogenate. SBT treatment on its own did not induce any significant change in the LPO level, whereas STZ treatment resulted in a significant ( $P< 0.001$ ) increase in MDA level in diabetic rats (group II). SBT treatment significantly ( $P< 0.001$ ) decreased the level of MDA in diabetic rats when compared with diabetic control rats. Lowering of LPO level was also observed in gliclazide treated diabetic rats.

#### 3.5. Effect of SBT on STZ – Induced Changes in GSH Content

Table 4 shows the contents of GSH animals treated with STZ, which was significantly ( $P<0.001$ ) decreased in pancreatic tissue as compared to the normal control rats (group-I). Pancreatic glutathione content increased significantly in SBT treated diabetic rats (group IV and V) when compared with diabetic control rats. Gliclazide has also replenished the GSH contents in STZ diabetic rats.

#### 3.6. Effect of SBT on STZ – Induced Histological Changes in Pancreas

STZ induced diabetes resulted in degenerative and lytic changes in the islets of Langerhans of pancreas (Fig. 1). The lobules of pancreatic acini were seen with areas of fibrosis. There was depletion of  $\beta$  cells and the dimension of the islet is also considerably reduced and shrunken. The histological picture of pancreas was improved in diabetic rats treated with SBT (Fig. 1), as evidenced by the regeneration of  $\beta$  cells, which reveals the protective effect of SBT.

### 4. DISCUSSION

Diabetes mellitus is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. Lipid peroxidation has been implicated in the pathogenesis of naturally occurring or induced diabetes [20]. There is a wide range of antioxidant defenses which protects against the adverse effects of free radicals production *in vivo* [21].

Disturbances of antioxidant defense systems in Diabetes mellitus have been reported [22]. Therefore, treatment with antioxidant may contribute to the prevention and delaying of diabetic complications [23]. The antioxidant activity of the

**Table 2. OGTT in Normal and Diabetic Rats Treated with Sea Buckthorn Fruit Pulp**

GRP	Treatment	0 min	30 min	60min	90 min	120 min
1	Normal saline (1 ml/kg, p.o.)	79.34± 3.05	120.94± 2.06	107.58± 2.70	102.3± 2.95	100.6±3.03
2	STZ (60 mg/kg, i.p.) + NIC (120 mg/kg, i.p.)	256.56±5.84	301.51±7.72	328.78±5.69	342.92±9.78	318.18±6.83
3	Sea buckthorn fruit pulp (2ml/kg, p.o.)	85.34±2.69	120.20±3.29	127.77±2.34	124.14±1.69	92.92±3.51
4	SBT (1ml, p.o.)+ glucose load	73.28± 1.50	114.35± 2.21	103.91± 3.01	101.8± 3.15	95.83±2.91
5	SBT(2 ml, p.o.) + glucose load	73.43± 0.99	92.99± 1.62*	89.97± 2.37**	82.49± .93**	78.97±1.59**
6	Gliclazide (25 mg/kg)+ glucose load	74.52± 0.84	85.38± 1.85**	81.68± 2.28**	76.70± 1.65**	72.35±1.28**

Data are expressed in Mean ± SEM, n=6, for Day 0 comparison were made vs Group 1(normal control)- \*\* statistically significance at p<0.01 and \* statistical significance at p<0.05.

**Table 3. Effect of Sea Buckthorn Fruit Pulp and Gliclazide on TBARS Thiobarbituric Acid Reactive Substances**

Group	Treatment	TBARS level (nM/mg of protein)
1	Normal saline (1ml/kg, p.o.)	0.197 ± 0.031
2	STZ (60 mg/kg, i.p.) + NIC(120mg/kg,I.P.)	1.17± 0.052**
3	Sea buckthorn fruit pulp (1 ml/kg, p.o.)	0.24 ± 0.03 <sup>#</sup>
4	STZ±NIC+SBT (1 ml/kg, p.o.)	0.52 ± 0.07 <sup>#,ns</sup>
5	STZ±NIC+SBT (2 ml/kg, p.o.)	0.44 ± 0.03 <sup>#,ns</sup>
6	STZ±NIC+Glic(25mg/kg, p.o.)	0.40 ± 0.04 <sup>#</sup>

Data are expressed in mean ± SEM, n=6, comparison were made vs Group 1(normal control)-\*\* statistical significance at p<0.01, vs Group 2(diabetic control)-# statistical significance at p<0.01 and vs Group 6(standard)- ns statistical significance at p<0.01.

**Table 4. Effect of Sea Buckthorn Fruit Pulp and Gliclazide on Pancreatic Glutathione**

Group	Treatment	Glutathione (µg/mg of protein)
1	Normal saline (1ml/kg, p.o.)	4.33± 0.12
2	STZ (60 mg/kg, i.p.) + NIC(120mg/kg,I.P.)	2.30 ± 0.10**
3	Sea buckthorn fruit pulp (1 ml/kg, p.o.)	3.93 ± 0.83
4	STZ±NIC+SBT(1 ml/kg, p.o.)	2.36 ± 0.41
5	STZ±NIC+SBT(2 ml/kg, p.o.)	3.22 ± 0.36 <sup>€</sup>
6	STZ±NIC+Glic(25mg/kg, p.o.)	3.67 ± 0.27 <sup>€€</sup>

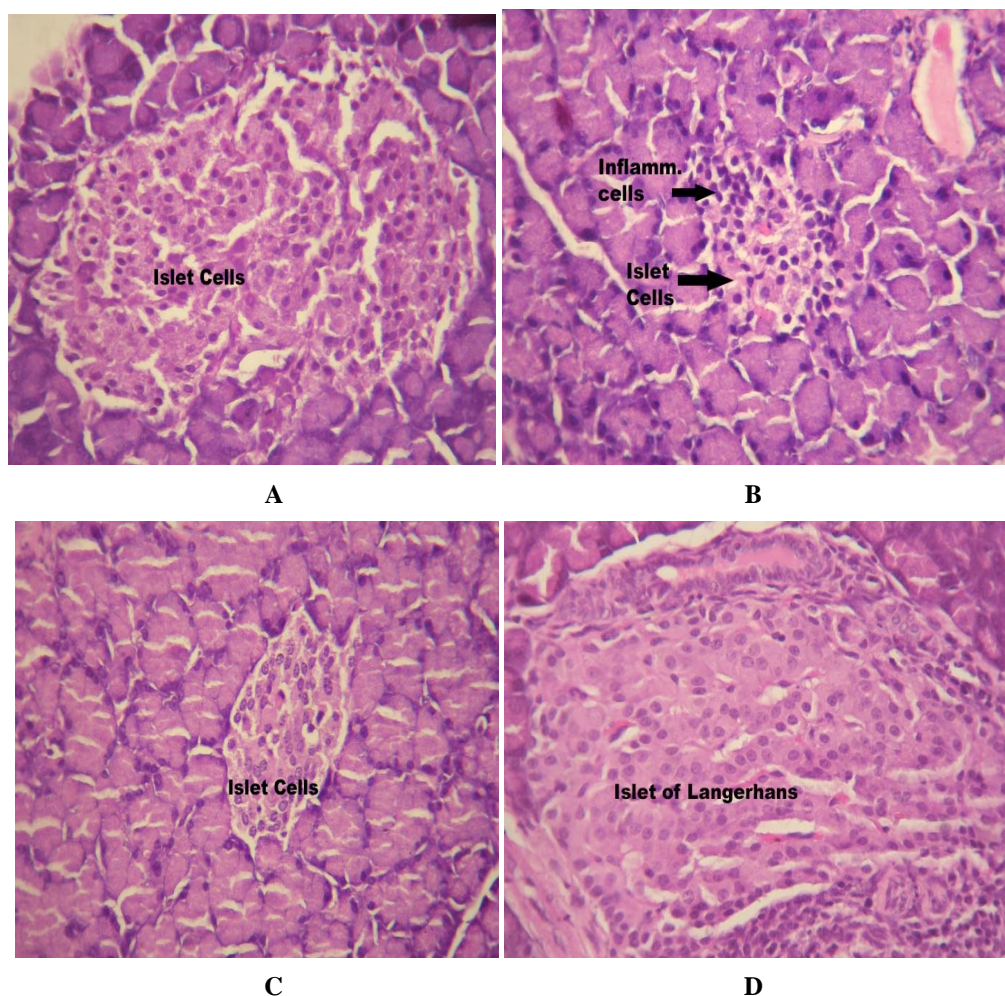
Data are expressed in mean ± SEM, n=6, comparison were made vs group 1(normal control) \*\* statistical significance at p<0.01 and vs group 2 (diabetic control)- € statistical significance p<0.05.

SBT fruit is due to the presence of high content of vitamin C and E, carotenoids, phytosterol, tannins as well as antioxidant enzymes [24]. STZ-diabetic rats exhibited persistent hyperglycemia. Seabuckthorn (SBT) treatment to STZ diabetic rats reduced the elevated blood glucose level thereby showing its antihyperglycaemic activity. In diabetes, there is an increased glycosylation of a number of proteins therefore measurement of HbA1c has proven to be particularly useful in monitoring the effectiveness of therapy

[25]. HbA1c level increased in STZ diabetic rats when compared to normal control rats.

Our results showed that SBT decreased blood glucose level, HbA1c level and improved glucose tolerance in diabetic rats. In a recent study antidiabetic effect of an aqueous extract from SBT seed residues has been demonstrated [12].

Some of the recent studies have shown the SBT has antioxidant properties and prevent LPO [5-7, 26]. Adminis-



**Fig. (1).** Hematoxylin and eosin- stained sections of rat pancreas: (A) Represents normal control group showing numerous beta cells with abundant basophilic cytoplasm. (B): Represents STZ treated rats showing atrophy of the beta cells. The beta cell cytoplasm is scanty and numerous inflammatory cells are seen in the upper part of the islet. (C) Represents pancreatic section of STZ + SBT (2ml) treated rats showing regeneration of beta cells with basophilic cytoplasm. No inflammatory cells are seen. (D) Represents pancreatic section of STZ+ GLC treated rats showing details of islet cells. The islet cells are of normal appearance (HE x 400).

tration of SBT to diabetic rats reduced the glycosylation of Hb by virtue of its free radical scavenging property and thus contribute to decreased level of glycated Hb in SBT treated diabetic rats.

SBT by its ability to scavenge free radicals and to inhibit lipid peroxidation prevents STZ-induced oxidative stress and protects  $\beta$ -cells resulting in decreased blood glucose levels. Cao *et al.*, 2003 [27] have also reported that the flavonoids from the seed and fruit residue of *Hippophae rhamnoides* L. exhibited hypoglycaemia and hypolipidemic effects. In the oral glucose tolerance test, SBT significantly reduced the blood glucose level in glucose loaded rats at 30, 60, 90 and 120 min. as compared with control rats, loaded only with glucose (Table 2). It has been reported in various studies that increased oxidative stress may play a role in pathogenesis and progression of diabetic tissue damage [28].

In our study, we observed a significant increase in the concentration of MDA, a secondary product of LPO, in pancreas of STZ treated animals. LPO induced by STZ was associated with the decreased enzymatic activities and non-enzymatic (GSH) antioxidant contents. Since an oxidative

stress affects the cellular integrity only when antioxidant mechanisms are no longer able to cope with the free radical generation, supplementation of an antioxidant could gear up the detoxification machinery.

SBT berries has been reported to be a rich source of vitamin A,C,E,K, flavonoids, carotenoids, organic acids and oils, was studied for its protective effect against oxidative stress induced by STZ. The cytotoxic effect of STZ in  $\beta$ -cells is mediated by ROS [2]. Drugs with antioxidant properties may supply endogenous defense systems and reduce both initiation and propagation of reactive oxygen species [29]. Results of the present study clearly showed that LPO level was decreased in SBT treated diabetic rats. These results suggest that SBT administration in diabetic rats reduce LPO possibly by decreasing free radical formation, increasing antioxidant and associated enzymes content with significant lowering in blood glucose level.

Reduced glutathione (GSH), the cellular antioxidant was lowered in animals treated with STZ. However, in diabetic animals co-treated with SBT, GSH level was increased suggesting protective effect on later by SBT. These findings

of elevation of GSH contents by SBT suggest GSH-dependent detoxification of free radicals. In addition, Paolisso *et al.*, 1992 [30] have proposed that the ratio of GSH/GSSG plays a critical role in the glucose homeostasis of diabetes. The effects of SBT on plasma glucose and pancreatic LPO produced by STZ may be related to the significant rise in pancreatic glutathione induced by this drug. Numerous studies indicate that dietary supplementation with antioxidant nutrients may be a safe and simple complement to traditional therapies for preventing and treating diabetic complications. Protective effect of SBT is probably due to the counteraction of free radicals by its antioxidant nature and is also evident from the histological observation of pancreas, which shows similar morphology as compared to the normal control rats. The diabetic rats in contrast however showed extensive degeneration and multiple necrotic changes. The present study thus confirms that pancreatic  $\beta$ -cell gets destroyed by STZ and SBT causes its regeneration. In conclusion, our present results demonstrated that SBT, a widely used plant with potent antioxidative activity, can exert anti-diabetic effects by preserving  $\beta$ -cell function in STZ diabetic rats. The protective effects of SBT could be attributed to several constituents with potentially healthy biological properties such as Vitamin C, carotenoids, tocopherols, flavonoids and phytosterols. Nevertheless, detailed mechanistic action on different free radicals by SBT particularly the flavonoids present in SBT requires further study.

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