Journal of Animal Research: v.6 n.5, p. 787-794. October 2016

DOI: 10.5958/2277-940X.2016.00099.1

Ameliorating Effect of Seabuckthorn Leaf Extract Supplementation on Streptozotocin Induced Diabetes Mellitus in Wistar Rats

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Received: 07 May, 2016 **Accepted:** 29 July, 2016

ABSTRACT

Study was conducted to evaluate the effects of Seabuckthorn leaf extract (SLE) supplementation on biochemical parameters in streptozotocin (STZ) induced diabetes mellitus in Wistar rats. Thirty two adult male Wistar rats were divided into four groups namely CON (negative control), SCO (Seabuckthorn control), DCO (Diabetic control), and DSL (Diabetic seabuckthorn treatment group). Diabetes mellitus was induced by single intra peritoneal injection of STZ @ 50 mg/kg body weight in DCO and DSL group of rats. SLE was administered orally @ 100mg/kg body weight for 40 days to SCO and DSL groups. CON served as the negative control. Blood samples were collected from experimental animals on zero, 20th, and 40th days of trial to study various biochemical parameters. Significantly ($P<0.01$) lower levels of total serum protein, and hepatic glycogen and significantly ($P<0.01$) higher serum glucose, total cholesterol, urea and creatinine levels were observed in DCO group in comparison to CON group. However, in SLE treated diabetic rats (DSL group) significant ($P<0.01$) improvement was observed in all the above parameters. It may be concluded that SLE exerts ameliorative effect over Diabetes mellitus induced biochemical alterations in Wistar rats.

Keywords: Streptozotocin, diabetes mellitus, seabuckthorn, wistar rats

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycaemia with consequent altered metabolism of carbohydrates, proteins and lipids. It is ever increasing problem in India with 50.8 million diabetics (Sharma *et al*., 2011). Diabetes is also a growing problem in dogs and cats and prevalence is increasing over time. Prolonged hyperglycaemia in diabetes mellitus is associated with a variety of diabetic complications (Hoffman *et al*., 2001), and pathological changes in various tissues/organs involving nervous system (Kamenov *et al.*, 2010), liver, kidney (Inukai *et al*., 2000), heart (Alan and Karin, 2009) and eyes (Romero-Aroca *et al.*, 2009).

Many pharmaceutical drugs are presently available for the treatment of diabetes mellitus (Matsui *et al*., 2006). However due to accompanying side effects, the use of these drugs are−restricted (Noor *et al*., 2008). Some of the plant extracts have long been used for the treatment of diabetes mellitus in various systems of medicine and are currently accepted as an alternative for diabetic therapy. Seabuckthorn *(Hippophae rhamnoides)* is a bush species widely distributed throughout the temperate zone of Asia, Europe and all over subtropical zones, being found especially at high altitudes. In India it has been reported to be found in high altitude regions of Himachal Pradesh, Jammu & Kashmir and Uttrakhand (Bhardwaj *et al.*, 2015). Seabuckthorn is abundant in nutrients and therapeutic compounds, such as flavonoids, carotenes, volatile oils, carbohydrates, vitamins, amino and mineral-acids (Sabir *et al*., 2005).

The leaves of the plant are rich in several nutrients and bioactive substances, mainly phenolic compounds including catechin, polyphenols, carotenoid lycopene, bioflavonoids, and coumarins (Saggu and Kumar, 2008; Kumar *et al.*, 2014; Dubey *et al.*, 2016). The leaves

also contain a significant concentration of vitamin C (Krejcarová *et al.*, 2015). Aqueous extract of seabuckthorn leaves reported to possess antidiabetic activity in *in vitro* studies (Bhardwaj *et al*., 2015). Some of the bioactive phenolic constituents, such as quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol and isorhamnetin have been reported to be present in SBT leaf extracts (Upadhyay *et al*., 2010). The proposed study therefore envisages investigating the effect of Seabuckthorn leaf extract on biochemical alterations in experimentally induced diabetes mellitus in Wistar rats.

MATERIALS AND METHODS

The study was conducted in the Division of Veterinary Physiology & Biochemistry, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-kashmir University of Agricultural Sciences and Technology- Jammu, R.S. Pura, J&K, India.

Animals and experimental design

The Wistar rats used in the present study were procured from Indian Institute of Integrative Medicine (IIIM), Council of Scientific and Industrial Research, (CSIR) Lab, Jammu, India. The study was conducted on 32 adult healthy Wistar male rats with a mean body weight of 177.8±12.6 gms. The animals were maintained under standard managemental conditions and were provided standard pelleted ration and clean drinking water *ad libitum.* The animals were acclimatized for 1 week and kept under constant observation during entire period of study. The animals were divided into four equal groups. Group CON served as negative control, group SCO served as SLE control, group DCO as diabetic control and in group DSL induction of diabetes was followed by SLE treatment for 40 days.

Ethical approval

The animals were treated humanely during the whole period of experimental study and the work was approved by the institutional Animal Ethics Committee vide No. 862/ac/04/CPCSEA on ethical standards in animal experimentation.

Experimental procedure and chemical analysis

Streptozotocin (STZ) solution was prepared in 0.1 M sodium citrate (pH 4.5). Diabetes mellitus was induced in DCO and DSL group of animals by single intraperitoneal injection of STZ @50 mg/kg bodyweight as per Gayathri and Kannabiran (2008). Diabetes mellitus induction was confirmed after 72 hours in all injected animals by estimating serum glucose level for hyperglycaemia.

Seabuckthorn leaves were collected from the Ladakh divison of Jammu & Kashmir in the month of August. The collected leaves were duly verified by the subject expert from University of Jammu, Jammu. After shade drying, leaves were pulverised to powder form and SLE was prepared by cold percolation method (Saggu *et al.*, 2007). Briefly dried leaf powder was soaked in distilled water (1:5w/v) at room temperature (25°C). After 24 hours the supernatant was collected and the residue was resoaked in fresh water. The process was repeated four times for complete extraction. The supernatants were pooled and filtered through muslin cloth and filtrate was then centrifuged at 8000g at 4°C. After centrifugation the supernatant obtained was concentrated in a rotary evaporator to obtain a semisolid consistency of SLE and stored at -20^{o}C for further use. SLE was given orally @ 100 mg/kg body weight as per Saggu *et al.* (2007) to animals in groups SCO and DSL group for 40 days.

Before the start of experiment (0th day), blood collection was made from retro-orbital fossa of only 8 randomly selected rats, so that the stress of blood loss may be limited to only these animals. Subsequently rats were divided into four groups and on 20th and 40th day of experiment, blood samples were collected from all the experimental animals. Blood was collected in clean and dry microcentrifuge tubes, allowed to clot and serum was separated for biochemical analysis. The collected serum samples were analyzed for total serum protein, albumin, globulin, albumin: globulin ratio, urea, creatinine, total cholesterol and glucose using Ranbaxy Fine Chemicals Ltd. (RFCL) diagnostic kits. At the end of the experiment, animals were slaughtered, liver was collected and hepatic glycogen was estimated as per the method of Seifter *et al*. (1950).

Statistical analysis

Statistical analysis was performed using generalized linear model analysis of variance (Snedecor and Cochran, 1994) and Duncan's multiple range test (Duncan, 1955).

RESULTS

In this study, STZ injection resulted in significant (P<0.01) lowering of total protein, and hepatic glycogen levels (Table 1 and Table 3) and significant (P<0.01) increase in blood glucose, total cholesterol, urea and creatinine levels (Table 2) in DCO group in comparison to CON group animals.

Total protein and albumin levels were found to be lowest in diabetic group. SLE treatment resulted in significant (P<0.01) improvement in both the parameters. Globulin and albumin: globulin ratio was comparable between different groups and no significant difference was observed among groups (Table 1). Serum glucose, total cholesterol, urea and creatinine levels were found to be significantly higher (P<0.01) in STZ treated diabetic rats compared to control group. In SLE treated diabetic rats significant (P<0.01) improvement was observed (Table 2) with respect to all the above parameters.

Hepatic glycogen levels were significantly (P<0.01) lower in STZ treated diabetic rats than control groups and showed significant (P<0.01) changes towards normal ranges following SLE treatment (DSL group). No significant difference (P>0.05) was observed between hepatic glycogen levels of SCO and DSL group of rats (Table 3).

DISCUSSION

In present study single intraperitoneal injection of STZ

Table 1: Effect of SLE supplementation on biochemical alterations in STZ induced diabetes mellitus in Wistar rats

Group	Period			Group Mean	P value		
	0th day**	20th day	40th day	± SEM	Group	Period	Group × Period
Total protein(g/dl)							
CON	5.84 ± 0.04	5.90 ± 0.04	6.04 ± 0.04	5.93^{c} ± 0.01			
SCO		5.64 ± 0.06	6.08 ± 0.02	5.85^{c} ± 0.02			
DCO		4.93 ± 0.05	4.51 ± 0.05	5.09^{a} ± 0.03			
DSL		5.10 ± 0.01	5.54 ± 0.05	5.59^{b} ± 0.02			
Period Mean ± SEM	5.84^{b} ± 0.04	5.39^{a} ± 0.02	5.54^{a} ± 0.02	5.59 ± 0.01	<0.01	<0.01	<0.01
Albumin(g/dl)							
CON	4.30 ± 0.03	4.32 ± 0.05	4.00 ± 0.05	4.20^{c} ± 0.01			
SCO		4.14 ± 0.03	4.37 ± 0.05	4.27^{c} ± 0.01			
DCO		3.28 ± 0.02	2.93 ± 0.06	3.50^{a} ± 0.03			
DSL		3.55 ± 0.06	4.21 ± 0.02	4.02^{b} ± 0.02			
Period Mean ± SEM	4.30^{b} ± 0.03	3.82^{a} ± 0.02	3.88^{a} ± 0.02	4.00 ± 0.01	<0.01	<0.01	<0.01
Globulin(g/dl)							
CON	1.54 ± 0.05	1.58 ± 0.02	2.04 ± 0.05	1.72 ± 0.02			
SCO		1.49 ± 0.07	1.70 ± 0.06	1.58 ± 0.02			
DCO		1.76 ± 0.04	1.57 ± 0.08	1.62 ± 0.02			
DSL		1.62 ± 0.08	1.61 ± 0.04	1.59 ± 0.02			
Period Mean ± SEM	1.54 ± 0.05	1.61 ± 0.01	1.73 ± 0.02	1.63 ± 0.00	>0.05	>0.05	>0.05
Albumin: Globulin							
CON	2.98 ± 0.11	2.79 ± 0.07	2.04 ± 0.07	2.60 ± 0.03			
SCO		2.35 ± 0.04	2.85 ± 0.13	2.73 ± 0.04			
DCO		1.89 ± 0.04	2.13 ± 0.18	2.34 ± 0.04			
DSL		2.61 ± 0.15	2.43 ± 0.03	2.67 ± 0.04			
Period Mean ± SEM	2.98 ± 0.11	2.41 ± 0.02	2.36 ± 0.03	2.58 ± 0.01	>0.05	>0.05	>0.05

(*Values with different superscripts with in a column/row differs significantly; ** n=8)

Table 2: Effect of SLE supplementation on biochemical alterations in STZ induced diabetes mellitus in Wistar rats

Group	Period			Group Mean ± SEM	P value		
	0^{th} day**	20^{th} day	40^{th} day		Group	Period	Group × Period
Glucose (mg/dl)							
CON	105.09 ± 1.70	116.76 ± 0.60	120.45 ± 0.62	114.10^{a} ± 0.45			
SCO		113.32 ± 0.89	118.24 ± 0.53	112.22^{a} ± 0.43			
DCO		294.12 ± 1.70	306.58 ± 0.88	235.27^{c} ± 3.95			
DSL		180.83 ± 1.13	150.63 ± 1.42	145.52^{b} ± 1.40			
Period Mean ± SEM	105.09^{a} ±1.70	176.26^{b} ± 2.34	173.98^{b} ± 2.47	151.78 ± 0.74	<0.01	<0.01	<0.01
Cholesterol (mg/dl)							
CON	83.35 ± 1.30	75.41 ± 0.95	69.15 ± 0.77	75.97^{a} ± 0.41			
SCO		81.04 ± 0.91	66.84 ± 0.48	77.07^{a} ± 0.44			
DCO		97.28 ± 1.14	99.73 ± 0.30	93.45^{c} ± 0.45			
DSL		89.20 ± 0.62	81.68 ± 0.29	84.74^{b} ± 0.30			
Period Mean ± SEM	83.35^{b} ±1.30	85.73^{b} ± 0.34	79.35^{a} ± 0.43	82.81 ± 0.12	<0.01	<0.01	<0.01
Urea (mg/dl)							
CON	16.81 ± 0.51	16.13 ± 0.43	16.51 ± 0.30	16.48^{a} ± 0.14			
SCO		16.14 ± 0.34	16.07 ± 0.32	16.34^{a} ± 0.13			
DCO		33.16 ± 0.26	43.37 ± 0.20	31.11^{c} ± 0.48			
DSL		28.35 ± 0.21	21.30 ± 0.26	22.15^{b} ± 0.23			
Period Mean ± SEM	16.81^{a} ± 0.51	23.44^{b} ± 0.25	24.31^{c}± 0.26	21.52 ± 0.09	<0.01	<0.01	<0.01
Creatinine (mg/dl)							
CON	0.66 ± 0.03	0.70 ± 0.03	0.57 ± 0.03	0.64^{a} ± 0.01			
SCO		0.63 ± 0.03	0.66 ± 0.02	0.65^{a} ± 0.01			
DCO		1.07 ± 0.03	2.52 ± 0.05	1.42^{c} ± 0.04			
DSL		0.89 ± 0.01	0.81 ± 0.02	0.78^{b} ± 0.01			
Period Mean ± SEM	0.66^{a} ± 0.03	0.82^{b} ± 0.01	1.14^{c} ± 0.03	0.87 ± 0.01	<0.01	<0.01	<0.01

(*Values with different superscripts with in a column/row differs significantly; ** n=8)

@ 50 mg/kg of body weight resulted in significant hyperglycemia (51.5%) in Wistar rats (DCO and DSL group) as compared to control animals. Similar observations were also reported by Rawi *et al*. (2011) and Kamalakkannan and Prince (2005).

These elevated blood glucose levels in STZ induced diabetic rats indicates insulin deficiency, due to partial destruction of insulin producing pancreatic β-cells by STZ (Aybar *et al*., 2002). Insulin is a polypeptide hormone produced by the beta cells of islets of Langerhans of pancreas. It is the principle hypoglycaemic hormone in the body which regulates blood glucose levels within physiological limits by promoting the glucose uptake and its utilization and by inhibiting its production in body.

A significant decrease in total protein and albumin concentration in diabetic rats was observed in comparison to control rats in our study. Similar findings have also been reported by Gupta *et al*. (2010) and Mahboob *et al.* (2005) in rat and human, respectively. These effects can again be explained in the light of insulin deficiency, increased lipid peroxidation (Kamalakkannan and Prince, 2005) and hepatotoxicity (Ravi, 1995; El-Demerdesh *et al*., 2005; Kim *et al*., 2006) in diabetes mellitus. Insulin being an anabolic hormone stimulates the entry of amino acids

into the cells, enhances protein synthesis and reduces protein degradation (Satyanarayana and Chakrapani, 2008). Additionally no significant difference in globulin levels and A:G ratio was observed. This seems to show the general well-being of animals with no exposure to any kind of infection during the experimental period.

Table 3: Effect of SLE supplementation on hepatic glycogen in STZ induced diabetes mellitus in Wistar rats

Group	Hepatic Glycogen (µg/g)
CON	6.23^{c} ±0.23
SCO	7.09^{b} ±0.13
DCO	4.26^{a} ±0.22
DSL	7.04^{b} ±0.17
P value	<0.01

*Values with different superscripts with in a column/row differs significantly.

There is a significant increase in total cholesterol levels (23%) observed in STZ induced diabetic rats compared to control rats in present study. These findings are in accord to the reports of several workers (Gupta *et al*., 2010; Rawi *et al*., 2011). Hassan (2007) has suggested the reduced rate of cholesterol removal from circulation for hypercholesterolemia in diabetes. Additionally uninhibited action of lipolytic hormones such as glucagon and catecholamines on fat depots, in the absence of insulin might also be credited for hyperlipidemic state in diabetes mellitus (Ravi *et al*., 2005).

In this study, significant increments in the levels of urea (89%) and creatinine (121%) were observed in STZ induced diabetic rats in comparison to control animals. Several studies have also shown an increased correlation between serum urea and creatinine in diabetic patients (Augusti and Sheela, 1996; Campos *et al*., 2003; Patel *et al*., 2009; Gupta *et al*., 2010). Although the elevated plasma levels of urea and creatinine are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988), this rise may be attributed to hyperglycemia induced osmotic diueresis and depletion of extracellular fluid volume (Patel *et al*., 2009). In diabetes mellitus the increased rate of protein catabolism due to the absence of insulin (Satyanarayan and Chakrapani, 2008), may also be the reason for elevated blood urea levels.

The significant decrease (32%) was observed in hepatic glycogen level in diabetic rats compared to control rats in present study. The results of this study corroborates with the findings of several other workers (Ahmed *et al*., 2006; Rawi *et al*., 2011). Despite of consistent hyperglycemia in diabetes mellitus, cells are not able to utilize glucose because of absence of insulin, leading to increased glucose output from the liver. The hepatic glycogen loss in diabetes mellitus might be attributed to the loss of insulin induced glycogen synthetase activating system (Huang *et al*., 2006) and/or altered activity of glycogen metabolizing enzymes in diabetic rats (Ahmed *et al*., 2006; Rawi *et al*., 2011).

Effect of SLE supplementation

In present study, the oral treatment of STZ induced diabetic rats with SLE @ 100 mg /kg body wt. resulted in significant lowering of blood glucose levels. These results are in accordance with study of Sharma *et al*. (2011) in which Seabuckthorn (SBT) fruit pulp treatment to STZ diabetic rats reduced the elevated blood glucose level. Antioxidant and tissue regenerative properties (Sharma *et al*., 2011) of SLE may be credited for normalization of insulin levels in diabetic animals, which might have resulted in protection of pancreatic β-cells from STZ induced oxidative stress and their regeneration. SBT has been reported to have capacity of β-cell regeneration (Sharma *et al*., 2011). The antidiabetic and antioxidant activity of SBT seeds (Zhang *et al*., 2010), fruit extracts (Sharma *et al*., 2011) and leaves (Bhardwaj *et al*., 2015) have been reported earlier. SBT leaves contain nutrients and bioactive substances which mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols (Saggu and Kumar, 2008; Kumar *et al*., 2014). The leaves are an equally rich source of important antioxidants including β-carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of calcium, magnesium and potassium (Suryakumar and Gupta, 2011). Several flavonoids present in SBT leaves may also be credited for the hypoglycemic activity. Plant flavonoid also been reported to have hypoglycemic potential (Bolkent *et al*., 2008). Cao *et al*. (2003) have reported the hypoglycaemia and hypolipidemic effects of flavonoids from the seed and fruit residue of *Hippophae rhamnoides* L. The flavonoid exert their effect by either promoting the entry of glucose into cells, stimulation of glycolytic enzymes

and glycogenic enzymes, depression of gluconeogenic enzymes or inhibiting the glucose-6-phosphatase in the liver, subsequently reducing the release of glucose in the blood (Naik *et al*., 1991). The hypoglycemic potential of SLE can also be due to its Poly Unsaturated Fatty Acids (PUFA) content (Yang and Kallio, 2002; Zheng *et al*., 2009) which is reported to increase the number of insulin receptors thus increasing the insulin activity (Hassan, 2007).

In this study, the oral treatment of STZ induced diabetic rats with SLE @ 100 mg/kg body wt. caused marked improvement of serum total protein and albumin contents in comparison to untreated diabetic rats. These results run parallel to the study of Kamalakkannan and Prince (2005) which reported that serum total protein concentration was increased in STZ-diabetic rats treated with different plants extracts. A good correlation between protein synthesis and insulin level has been observed by El-Shenawy and Nabi (2006). Therefore, this improvement could be attributed to increased protein synthesis, increasing incorporation of certain amino acids as a result of increasing insulin secretion, increase of hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme catalysing amino acids transamination.

SLE treated diabetic rats also showed decreased serum urea and creatinine levels in comparison to non-treated diabetic animals, which may be again be correlated with hypoglycaemic and antioxidant properties of SBT leaf extract. Patel *et al.* (2009) has correlated the hyperglycaemia and oxidative stress to the occurrence of nephropathy. Insulin secretory action of quercetin (Nuraliev and Avezov, 1992), one of the flavonoid present in SBT leave extract (Upadhay *et al.,* 2010) may also have resulted in shifting of protein metabolism from catabolic stage to anabolic stage and concomitant decrease in elevated urea and creatinine levels, thus showing the protective role of SLE in diabetes induced nephropathy.

In this study SLE treatment resulted in significant reduction of total cholesterol in diabetic rats. Again the normalization of insulin levels by SLE treatment might have played the role for its hypocholesterolemic effects in diabetic rats. Plant flavonoids have been reported to facilitate lipid metabolism in diabetics thereby lowering cholesterol levels (Fernandes *et al*., 2009). Naringerin (plant flavanoid) lowers serum cholesterol levels by inhibiting the activity of HMG-CoA reductase (Bok *et al*., 1999) which is an important enzyme for cholesterol biosynthesis. The polyunsaturated fatty acid (PUFA) content (Suryakumar and Gupta, 2011) of SBT may also be responsible for its cholesterol lowering effects (An *et al*., 1995). Leaves of SBT are rich in flavonoids, particularly quercetin, tannins, phenols and triterpenes which are responsible for its antioxidant activity. The ability of quercetin to reduce plasma cholesterol and triglycerides could be explained by its insulin releasing capacity (Nuraliev and Avezov, 1992). Also the ability of scavenging free radicals and antioxidant properties of the SBT leaf extract (Bhardwaj *et al*., 2015) may also participates in the hypolipidemic activity by inactivating hepatic HMG-CoA reductase, a key enzyme, in cholesterol synthesis. Jung *et al*. (2006) stated that, flavonoids decreases liver HMG-CoA reductase activity in type II diabetic mice.

Liver glycogen level may be considered as the best marker for assessing anti-hyperglycaemic activity of any drug (Grover *et al*., 2000). Enhanced glycogen breakdown/ glycogenolysis (Hassan, 2007) and decreased glucokinase activity may explain the decreased ability of the diabetic liver to accumulate glycogen (Rawi *et al*., 2011). Protection and regeneration of insulin producing cells by SLE might be the reason for improved glycogen content in diabetic liver in our study (Sharma *et al.*, 2011). Increased glycogen synthesis has been reported by flavonoids in hepatocytes of STZ diabetic rats (Fernandes *et al.,* 2009). Normalization of insulin levels in turn promotes conversion of inactive form of glycogen synthatase to active form and enhances conversion of blood glucose into glycogen (Rawi *et al*., 2011).

CONCLUSION

SLE treatment of diabetic rats@ 100mg/kg BW resulted in significant normalization of blood glucose, total protein, albumin, urea, creatinine, total cholesterol and hepatic glycogen levels in streptozotocin induced diabetic rats.

ACKNOWLEDGEMENTS

The authors are highly thankful to Dean, Faculty of Veterinary and Animal Science SKUAST-J, R.S Pura Jammu for providing necessary facilities. The authors are greatful to Indian institute of Integrated medicine, Council

of scientific and industrial research Laboratory, Jammu for providing experimental rats. The trial was carried out at Division of Biochemistry, FVSc & AH, SKUAST-Jammu.

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