

Original Research Article

Investigation of anti-diabetic properties of *Bridelia stipularis* L. (Leaves) on alloxan induced Type-2 diabetic rats.

Abstract

Background: Type 2 diabetes mellitus (T2DM) is a chronic disease that is associated with insulin secretion, insulin action or both. Its development is directly connected with not only carbohydrate metabolism but also primarily on lipid metabolism. Oral hypoglycemic agents have been found with some serious complications which are major clinical problems. Treatment of DM with medicinal plants and plant based traditional medicine is a potential adjunct therapy to maintain better glycemic control with a fewer side effects. The present study investigated the antidiabetic effect of *Bridelia stipularis* L. leaves on alloxan induced type-2 diabetic rats.

Methods: Diabetes was induced by a single dose of intraperitoneal injection of alloxan (150mg/kg) in SD rats of either sex and was divided into 5 groups of 6 animals each. Ethanolic extract of leaves from *Bridelia stipularis* (BS 250 and 500mg/kg) and glibenclamide (10mg/kg) were orally administered once daily for 21 days in the treatment and standard group respectively. Blood glucose levels were measured on 0, 7, 14 and 21 days of oral treatment. OGTT was performed on type-2 diabetic rats and at the end of the experiment, rats were sacrificed and blood samples were collected for the measurement of total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL) and high density lipoproteins (HDL), S. creatinine ALT and AST levels.

Results: Result of acute study (OGTT) showed significant effect ($p < 0.001$) after 120 minutes at 500mg/kg dose. After 21 days treatment with BS at 500mg/kg demonstrated a significant improvement ($p < 0.001$) in the blood glucose level. The BS extract also significantly lowered TC ($p < 0.001$), TG ($p < 0.001$), LDL-C ($p < 0.001$) levels and improved HDL-C insignificantly in hyperglycemic animals. Oral administration of BS significantly reduced the serum creatinine ($p < 0.001$), ALT ($p < 0.001$) and AST ($p < 0.001$) levels when compared to the vehicle treated diabetic control group. The standard drug (glibenclamide) showed significant effect ($p < 0.001$) on all the parameters. These findings ensured marked improvement of the pancreatic islet cells indicating antidiabetic effect of BS extract.

Conclusion: Our observations strongly suggest that ethanol extract from the leaves of *B. stipularis* has antidiabetic properties, which is mediated by hypoglycemic, hypolipidemic, and

hepatoprotective effects. Further studies are suggested to investigate the mechanisms of antidiabetic action of the plant.

Keywords: *Bridelia stipularis*, hypoglycemic effect, hypolipidemic effect, alloxan induced diabetes, type-2 diabetes mellitus.

Introduction

Diabetes Mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycemia with deficiency of secretion or action of endogenous insulin and no definite cause (Robles GI and Singh-Franco D, 2009; Paik SG, Blue ML, Fleischer N and Shin SI, 1982 and Kataoka S *et. al.*, 1983). It is a multifactorial illness with lipoprotein abnormalities, high basal metabolic rate, and high oxidative stress induced damage (Scoppola A, Montecchi FR, Menzinger G and Lala A, 2001; Owu DU *et. al.*, 2006; Ahmed ABA, Rao AS and Rao MV, 2010). Chronic hyperglycemia may lead to complications of diabetes like changes in metabolism, nerve, kidney, foot ulceration, and vascular tissue. Protein glycation, the most important source of free radicals, contributes to the progression of these complications in both types 1 and 2 diabetes and mediates the pathogenic effects (Yim G, Wang HH and Davies J, 2007; Evans JL, Goldfine ID, Maddux BA and Grodsky GM, 2003).

Many therapeutic approaches have been utilized for treatment of diabetes including insulin and oral hypoglycemic agents. Most of the drugs in current use have been reported with serious side effects and cost of treatment is high (Modak M, Dixit P, Londhe J, Ghaskadbi S and Devasagayam TPA, 2007). Therefore, to face these challenges, plants can be used as the major source of drugs for the treatment of diabetes mellitus (DM) which have been used in Indian medicine and other ancient systems in the world for a long time (Akhtar FM and Ali MR, 1984). World ethnobotanical information about medicinal plants reports that almost 800 plants could be

used to control DM (Akhtar FM and Ali MR, 1984; Pepato MT, et. al., 1998). The World Health Organization (WHO) estimates that 80% of the worlds' populations use traditional medicine. The continued use of traditional medicines is linked to their low cost and a general belief that they have minimal side effects (Somani R, Kasture S and Singhai AK, 2006). The biodiversity of flora of Bangladesh is very broad and several native Bangladeshi medicinal plant species have a long tradition of use with great phytotherapeutic potential (Karunakar Shukla. 2009). So, research in medicinal plants is a vital sector for the discovery of promising drugs in Bangladesh (Mia AW and Ghani A, 1990).

Bridelia stipularis L. Blume locally known as Harinhara and Pat Khowi is a perennial evergreen woody climber or scandent shrubs, branches up to 20 m, rarely small trees. It is widely distributed to China, Taiwan, Bangladesh, India, Nepal, Sri Lanka, Myanmar, Thailand, Vietnam, Indonesia, Malaysia and Philippines. It is harvested from the wild for local use as food, medicine and source of materials. The plant is used in pleurisy & exudation, cough, fever, asthma, sores in mouth, jaundice, anaemia due to pregnancy, white spots in the skin, inflammation, diarrhea, hypertension and hyperglycaemia (Dressler, S. 1996; USDA, 2015; Useful Tropical Plants, 2015 and Flowers of India, 2015).

The aim of the present study was to evaluate the antidiabetic effect of *Bridelia stipularis* leaves against alloxan induced type-2 diabetic rats.

MATERIAL AND METHODS

Plant collection and Extraction

The leaves of *Bridelia stipularis* were collected, taxonomically identified and authenticated by the Bangladesh National Herbarium, Mirpur, Dhaka. The collected materials were shed dried at

35° – 40° C for a week and crushed into moderately coarse powder. This powder was extracted using ethanol, dried under reduced pressure and finally extract was obtained.

Experimental Animals

The study was conducted with adult Sprague Dawley (SD) rats (weighing 150-200g) of either sex. They were bred at the Jahangirnagar University animal house maintained at a constant room temperature of $22\pm 5^{\circ}\text{C}$, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure when animals were used after 12 hrs fasting. The rats had no access to food during the whole period of blood sampling. All protocols for animal experiment were approved by the institutional animal ethical committee.

Induction of Type 2 Diabetes

Rats were injected with a freshly prepared solution of alloxan monohydrate (i.p.) in saline (300 mM NaCl) at a dose of (150 mg/kg, b.w.). Alloxan injection can provoke fatal hypoglycemia as a result of reactive massive release of pancreatic insulin, so rats were also given orally 5–10 mL of a 20% glucose solution after 6 h. Rats were then kept for the next 24 h on a 5% glucose solution as beverage to prevent too severe hypoglycemia. After 1 week, rats displaying fasting glucose level 8-15 mmol/l were chosen for the experiments (Ben Abdallah KR, *et. al.*, 2015 and Leila Z *et. al.*, 2007).

107 Acute study

108 OGTT was conducted in control and treated groups of rats, 24 h before decapitation of rats. All
109 groups were administrated glucose (3g/kg) by gastric gavages route. Blood glucose levels were
110 determined at 0, 60 and 120 min subsequently to receive glucose and fasting glucose was
111 measured (Jarald E, Joshi SB, and Jain DC, 2009).

112

113 Chronic study

114 Hyperglycaemic (Type-2) animals were then divided into five groups of six animals each. Group
115 I and II were treated with saline and served as normal control and diabetic control. Group III was
116 administered glibenclamide and served as standard. Group IV and V were administered ethanolic
117 extract of *Bridelia stipularis* at 250mg/kg and 500mg/kg body weight by oral route. All doses
118 were continued for 21 days in hyperglyceamic rats. Blood samples were collected from the cut
119 tip of the tail at 0, 7, 14 and 21st day from the respective start of treatments and measured serum
120 glucose. At the end of the experiment rats were sacrificed, blood was collected and serum lipid
121 profile, creatinine, ALT and AST levels were estimated by enzymatic colorimetric method (Shah
122 NA and Khan MR, 2014).

123

124 Biochemical analysis

125 Serum glucose was measured by glucose-oxidase-peroxidase method (GOD-POD) using a
126 commercial kit (glucose kit, Randox™, UK). The total cholesterol, triglyceride (TG), HDL and
127 LDL by enzymatic-colorimetric method (Ramadan BK, Schaalan MF and Tolba AM, 2017).

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Statistical analysis

Graphs were prepared by using MS Excel 2007 and data analysis for animal studies were done by SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA) using the One way ANOVA followed by Bonferroni's post hoc test. All the data were presented as Mean $\pm$ SEM. *(P<0.05), **(p<0.01) and ***(p<0.001) were counted as significant, highly significant and very highly significant respectively as compared to the vehicle treated diabetic control group (Ramadan BK, Schaalaa MF and Tolba AM, 2017).

Result and Discussion

Diabetes mellitus is a chronic disease which causes millions of deaths worldwide each year as a result of the associated complications (International Diabetes Federation, 2013). Hyperglycaemia is an independent risk factor in the development of chronic diabetic complications. Therefore the management of type 2 diabetes relies on the maintenance of blood glucose concentration in a normal or near normal level (Sheard NF *et. al.*, 2004).

The present study investigated the effects of a medicinal plant, *Bridelia stipularis* (BS) on body weight, blood glucose, serum lipids, serum creatinine, SGPT and SGOT in alloxan induced type-2 diabetic model rats.

Treatment of diabetic rats with 250mg/kg and 500mg/kg of *B. stipularis* extract in the oral glucose tolerance test (OGTT), improved glucose tolerance at 120 minute which was found to be significant (p<0.001) at 500mg/kg. Glibenclamide (5mg/kg) showed a significant fall in serum glucose level at 120 min (p<0.001). Therefore, the extract of *B. stipularis* showed significant

antihyperglycemic effect at 120 min in fasting rats as well as when fed simultaneously with oral glucose load in type 2 model rats (table 1 and figure 1).

Table 1: Effect of ethanolic extract of BS on the OGTT in type-2 diabetic rats.

Group	Glucose level (mmol/l) (Mean±SEM)		
	0 minute	60 minutes	120 minutes
Normal Control	5.05±0.13	7.85±0.63	7.07±0.41
Diabetic Control	10.70±0.52	13.47±0.58	12.87±0.52
STD (Glibenclamide 5mg/kg)	10.68±0.59	9.93±0.67***	6.85±0.13***
BS 250mg/kg	10.96±0.60	10.70±0.50*	9.52±0.49**
BS 500mg/kg	10.52±0.42	9.87±0.42**	8.07±0.30***

[N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to diabetic control].

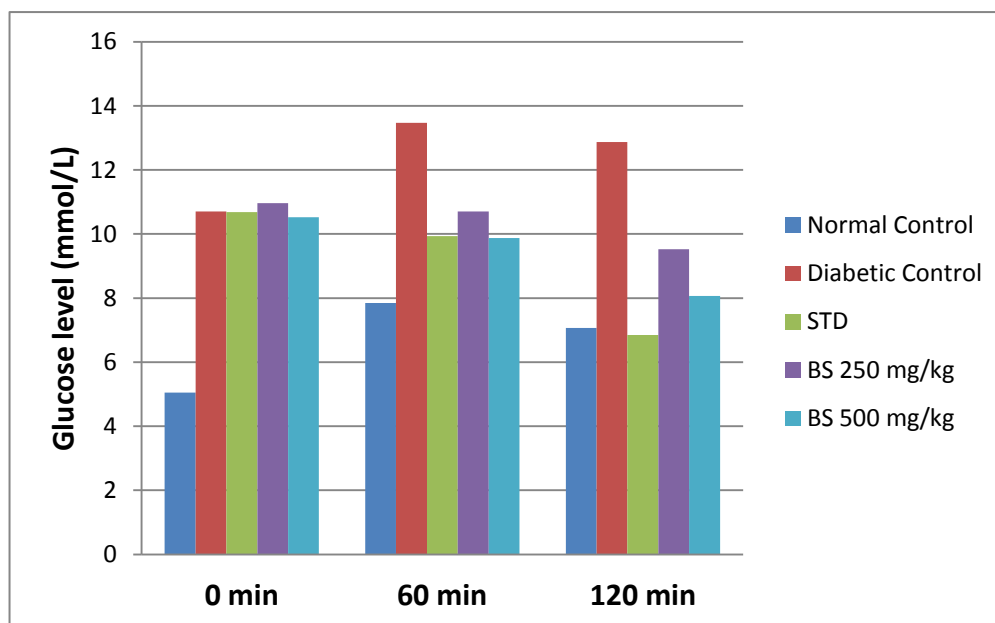


Figure 1: Effect of ethanolic extract of BS on the OGTT in type-2 diabetic rats.

Table 2: Effect of ethanolic extract of BS on the body weight after 21 days feeding in alloxan induced type-2 diabetic rats.

Group	Body Weight (gm) (Mean±SEM)	
	Day 0	Day 21
Normal Control	165.83±3.95	209.83±4.34
Diabetic Control	150.33±3.91	121.00±3.28
STD (Glibenclamide 5mg/kg).	152.0±3.01	178.00±2.65***
BS 250mg/kg	153.33±2.45	158.33±2.84**
BS 500mg/kg	155.13±1.70	167.83±2.21***

[N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6.
*(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to diabetic control.

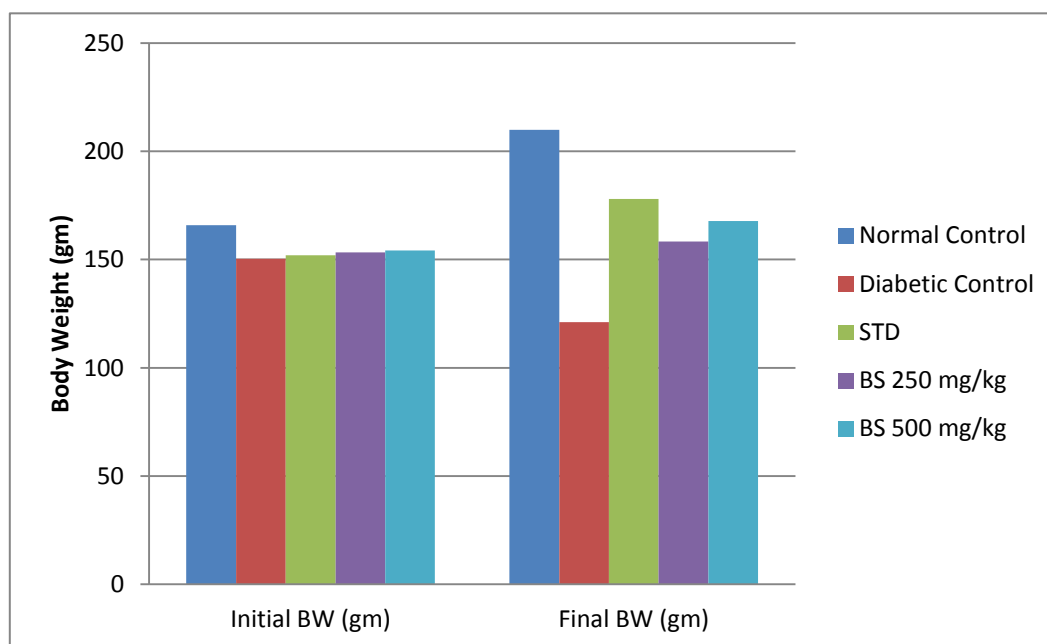


Figure 2: Effect of ethanolic extract of BS on the body weight after 21 days feeding in alloxan induced type-2 diabetic rats.

Table 3: Effect of ethanolic extract of BS on the fasting blood glucose level after 21 days feeding in type-2 diabetic rats.

Group	Glucose level (mmol/l) (Mean±SEM)			
	Day 0	Day 7	Day 14	Day 21
Normal Control	5.25±0.31	5.42±0.13	5.48±0.16	5.62±0.16
Diabetic Control	11.00±0.70	14.53±0.31	15.33±0.32	15.28±0.26
STD (Glibenclamide 5mg/kg)	10.37±0.56	9.47±0.43***	5.98±0.49***	5.30±0.16***
BS 250mg/kg	11.02±0.33	13.85±0.29	13.88±0.79	13.00±0.24**
BS 500mg/kg	10.80±0.49	13.95±0.40	11.68±4.32*	11.88±0.29***

[N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. * (p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to diabetic control].

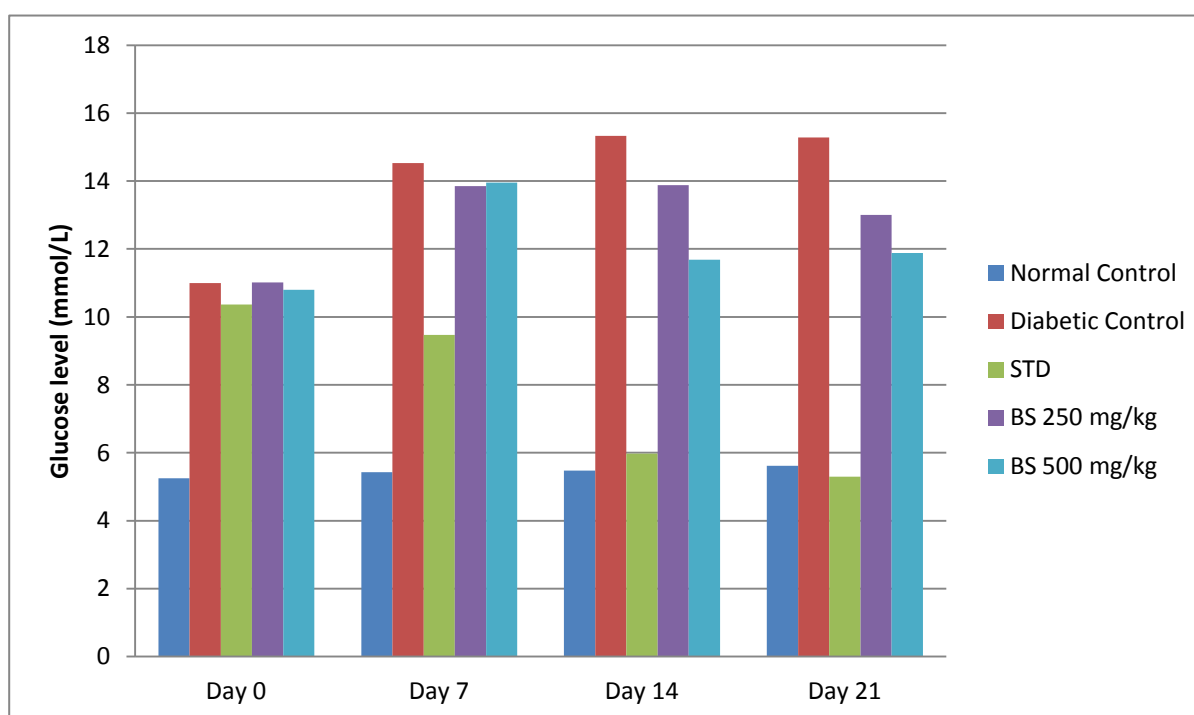


Figure 3: Effect of ethanolic extract of BS on the fasting blood glucose level after 21 days feeding in type-2 diabetic rats.

Table 4: Effect of BS on total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C level after 21 days feeding in type-2 diabetic rats.

Group	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal Control	91.33±2.69	70.50±0.88	38.83±2.27	32.17±1.64
Diabetic Control	193.33±7.69	178.17±3.35	123.83±2.27	19.33±1.76
STD (Glibenclamide, 5mg/kg)	97.66±3.03***	81.67±2.35***	48.50±1.91***	26.17±2.12
BS 250mg/kg	136.50±4.44**	124.33±5.04**	97.83±5.51**	21.50±1.83
BS 500mg/kg	111.83±4.85***	106.67±4.07***	80.50±3.73***	24.67±1.38

[N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to diabetic control.]

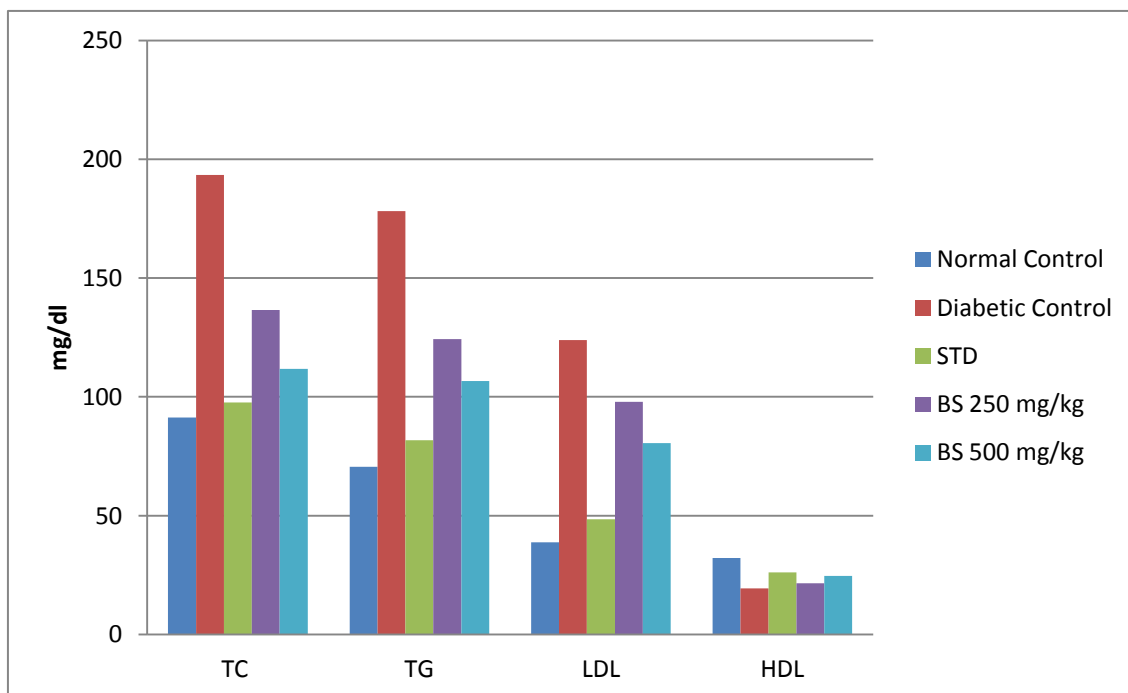


Figure 4: Effect of BS on total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C level after 21 days feeding in type-2 diabetic rats.

Table 5: Effect of BS extract on the serum creatinine level after 21 days feeding in type-2 diabetic rats.

Group	Serum creatinine level (mg/dl)
Normal Control	0.58±0.05
Diabetic Control	1.78±0.15
STD (Glibenclamide 5mg/kg)	0.61±0.08***
BS 250mg/kg	0.94±0.08**
BS 500mg/kg	0.69±0.04***

[N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to diabetic control.]

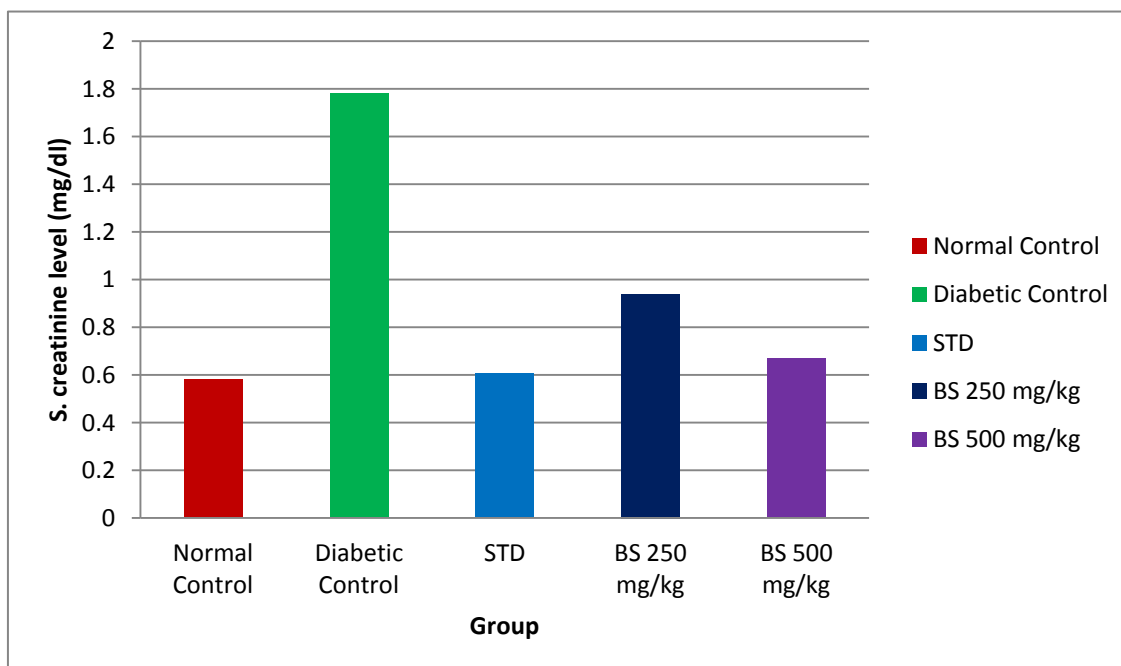


Figure 5: Effect of BS extract on the serum creatinine level after 21 days feeding in type-2 diabetic rats.

Table 6: Effect of BS extract on the SGPT and SGOT level after 21 days feeding in type-2 diabetic rats.

Group	SGPT (IU/L)	SGOT (IU/L)
Normal Control	45.00±2.96	41.33±1.71
Diabetic Control	117.33±2.60	86.33±3.44
STD (Glibenclamide 5mg/kg)	72.00±1.90***	47.50±2.20***
BS 250mg/kg	83.17±3.20***	70.33±3.21**
BS 500mg/kg	76.33±2.20***	52.50±2.17***

[N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to diabetic control. .

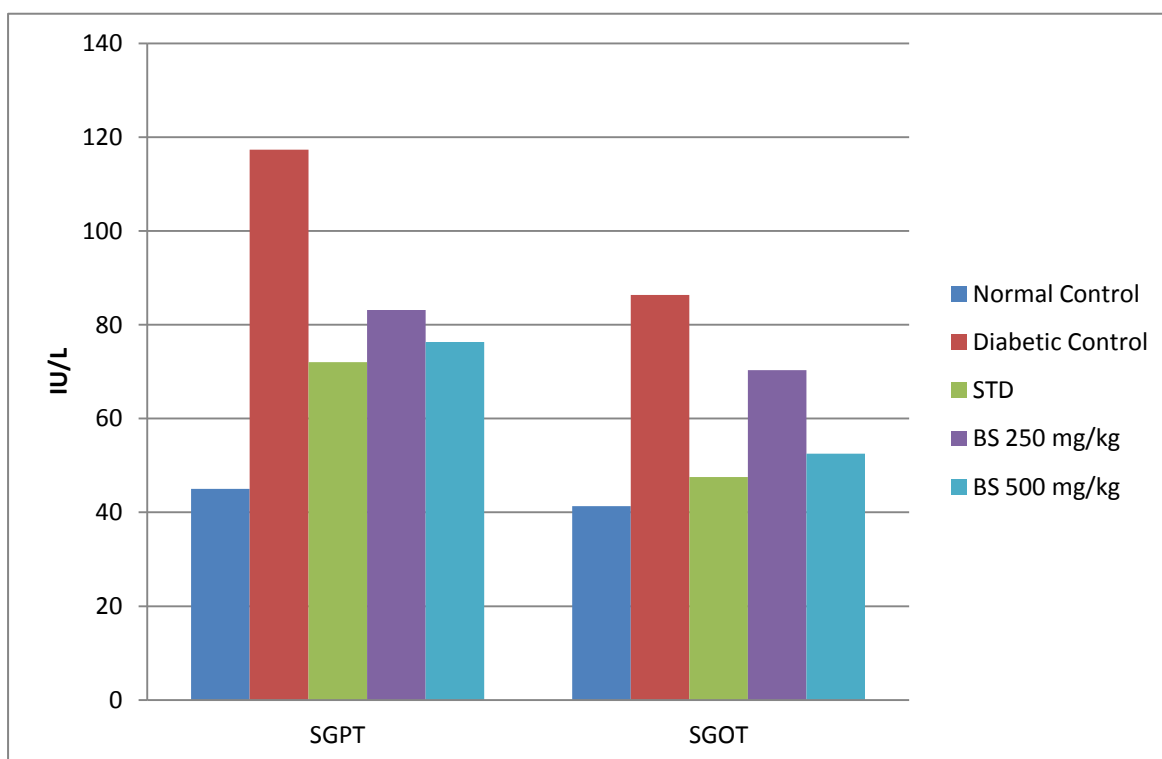


Figure 6: Effect of BS extract on the SGPT and SGOT level after 21 days feeding in type-2 diabetic rats.

227 It has been demonstrated that the post-prandial hyperglycemia is an important cardiovascular
228 risk factors in type-2 diabetes (Paolisso *et. al.*, 2003). Studies have shown that the post-meal
229 hyperglycemia doubled the risk of heart disease and fatal cardiovascular diseases (Barrett-
230 Connor & Ferrara 1998). In acute test the extract opposed the rise of blood glucose when was
231 fed with simultaneous glucose load.

232 The effect of ethanolic extract of BS (250mg & 500mg/kg) and standard antidiabetic drug
233 (glibenclamide 5mg/kg) on blood glucose level and other biochemical parameters in alloxan
234 induced type 2 diabetic rats has been depicted after 21 days continuous treatment.

235 It is known that loss of body weight and decreased growth rate in diabetic rats, is due to
236 increased catabolism of protein and muscle wasting (Eleazu CO *et. al.*, 2013). Rats treated with
237 BS extract improved body weight suggesting that the higher dose of BS could be protective
238 against protein degradation by improving glycemic control (table 2 and figure 2).

239 At day 0 and day 7 there was no comparable changes in blood glucose level as compared to
240 diabetic control group. At day 14 a significant change was observed by BS extract but highly
241 significant changes by glibenclamide (5mg/kg).

242 After a 21 days chronic study, the BS extract at both 250mg and 500mg/kg dose on type 2
243 diabetic rats showed highly significant reduction in serum glucose level ($p < 0.001$) whereas,
244 glibenclamide (5mg/kg) showed very highly significant ($p < 0.001$) reduction of glucose level.
245 Therefore, the extract has comparable antidiabetic activity with glibenclamide (table 3 and
246 figure 3).

Thus, the result of chronic antihyperglycemic study on alloxan induced type 2 diabetic rats indicates that, the BS decreases the serum glucose level highly significantly and in a time dependent manner.

Dyslipidemia is one of the complications of hyperglycemia. Untreated diabetic animals showed a significant increase in serum TC, LDL-C and TG concentrations against low levels of HDL-C after alloxan administration (Musabayane CT, Mahlalela N, Shode FO and Ojewole JA, 2005; Ravi K, Rajasekaran S and Subramanian S, 2005; Sharma SB, Balomajumder C and Roy P, 2008). The serum lipid profile of rats was evaluated in this study. Treatment of type 2 diabetic rats with ethanolic extract of BS (250 mg & 500mg/kg), improved dyslipidemia. At day 21, BS 500mg/kg produced significant reduction of total cholesterol ($p<0.001$), triglycerides ($p<0.001$), and LDL-C ($p<0.001$) level whereas HDL-C level was changed insignificantly as compared to the diabetic control group. The effects were comparable to that of the standard drug glibenclamide (table 4 and figure 4).

These ameliorating effects demonstrated the antihyperlipidemic effect of *B. stipularis*, and it could also be suggested that this antihyperlipidemic effects of BS pass through a decrease in intestinal cholesterol absorption or a decrease in the biosynthesis of cholesterol specifically by decreasing the activity of HMG-CoA reductase inhibitors (Sharma SB *et. al.*, 2003).

Hyperglycaemia is considered as major risk in the development of diabetic nephropathy. There are different mechanisms by which increased blood glucose level causes nephropathy. It produces the oxidative stress (Ha H, Kim C, Chung MH and Kim KH, 1994). Serum creatinine level is a biomarker of renal function.

269 Oral administration of *B. stipularis* extract reduced the serum creatinine level at both doses but
 270 significantly ($p < 0.001$) at 500mg/kg dose (table 5 and figure 5). A decrease in creatinine level by
 271 BS extract suggests an improvement in renal function and reinforcement from oxidative stress.

272 Increase in the plasma SGPT and SGOT are observed in the condition in which pancreas, liver,
 273 kidney and heart are destroyed (Fehling P *et. al.*, 1970). The present results showed that
 274 injection of alloxan induces a hepatocellular damage, which is indicated by significant increase
 275 in SGPT and SGOT in diabetic group as compared to control group (table 6 & figure 6).

276 In the present study, ethanolic extract of *B. stipularis* significantly decreased SGPT and SGOT
 277 enzyme level in diabetic rats. The improvements in the levels of the enzymes studied are a
 278 consequence of an improvement in the carbohydrate, fat and protein metabolism (Sunil C *et. al.*,
 279 2009). Therefore, BS treatment showed significant hepatoprotective activity in alloxan inuced
 280 type-2 diabetic rats.

281 In the present study, the *Bridelia stipularis* leaves were selected for the evaluation of possible
 282 antidiabetic activity. The above results suggest that, it has antihyperglycemic activity both at
 283 acute and chronic study. The lipid profile was found to be significantly improved. The alcoholic
 284 extract also has prominent effect on serum creatinine, SGPT and SGOT level.

285 **Conclusion**

286 The overall findings of this study focused that treatment of alloxan-induced diabetic rats, with
 287 the extract of *Bridelia stipularis* for 21days, could restore normal bioactivities by shifting lipid
 288 and carbohydrate metabolism homeostasis. Furthermore, BS showed significant nephroprotective
 289 and hepatoprotective actions. Therefore, it can be concluded that alcoholic extract of this plant

can be successfully utilized for the management of diabetes. In future, it is recommended to determine the mechanism(s) involved and, to identify the responsible active compounds

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