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Investigation of Anti-diabetic Properties of Ethanol Leaf Extract of *Bridelia stipularis* L. on Alloxan Induced Type-2 Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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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

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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.

1. INTRODUCTION

Diabetes Mellitus (DM) is a heterogeneous metabolic disorder characterised by hyperglycemia with deficiency of secretion or action of endogenous insulin and no definite cause [1,2,3]. It is a multifactorial illness with lipoprotein abnormalities, high basal metabolic rate, and high oxidative stress induced damage [4,5,6]. 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 [7,8].

Many therapeutic approaches have been utilised for the 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 [9]. 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 [10]. World ethnobotanical information about medicinal plants reports that almost 800 plants could be used to control DM [10,11]. 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 [12]. 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 [13]. So, research in medicinal plants is a vital sector for the discovery of promising drugs in Bangladesh [14].

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, diarrhoea, hypertension and hyperglycaemia [15,16,17].

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

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The leaves of *Bridelia stipularis* was collected from Sylhet area of Bangladesh, taxonomically identified and authenticated by the Bangladesh National Herbarium, Mirpur, Dhaka (Voucher No.

DACB 40630). The collected materials were shed dried at 35ºC–40ºC for a week and crushed into moderately coarse powder. This powder was extracted using ethanol, dried under reduced pressure and finally extract was obtained.

2.2 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±5ºC, 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.

2.3 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 [18,19].

2.4 Acute Study

OGTT was conducted in control and treated groups of rats, 24 h before decapitation of rats. All groups were administrated glucose (3g/kg) by gastric gavages route. Blood glucose levels were determined at 0, 60 and 120 min subsequently to receive glucose and fasting glucose was measured [20].

2.5 Chronic Study

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

2.6 Biochemical Analysis

Serum glucose was measured by glucose-oxidase-peroxidase method (GOD-POD) using a commercial kit (glucose kit, Randox™, UK). The total cholesterol, triglyceride (TG), HDL and LDL by enzymatic-colorimetric method [22].

2.7 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±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 [22].

3. RESULTS AND DISCUSSION

Diabetes mellitus is a chronic disease which causes millions of deaths worldwide each year as a result of the associated complications. 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 [23,24].

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 (Fig. 1).

It has been demonstrated that the post-prandial hyperglycemia is an important cardiovascular risk factors in type-2 diabetes [25]. Studies have shown that the post-meal hyperglycemia doubled the risk of heart disease and fatal cardiovascular diseases [26]. In acute test the extract opposed the rise of blood glucose when was fed with simultaneous glucose load.

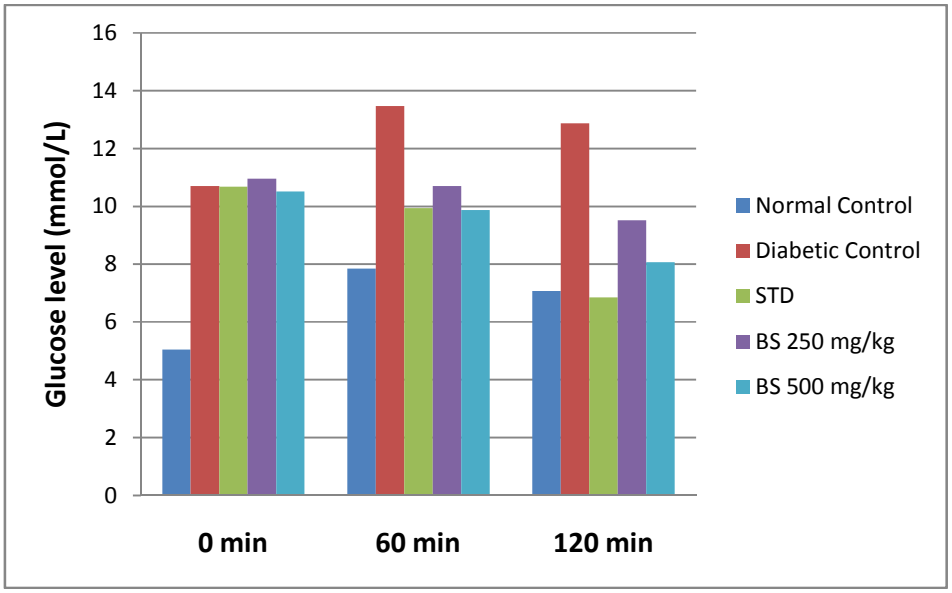

Fig. 1. Effect of ethanolic extract of BS on the OGTT in type-2 diabetic rats

*[N.B: Data were analysed 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]*

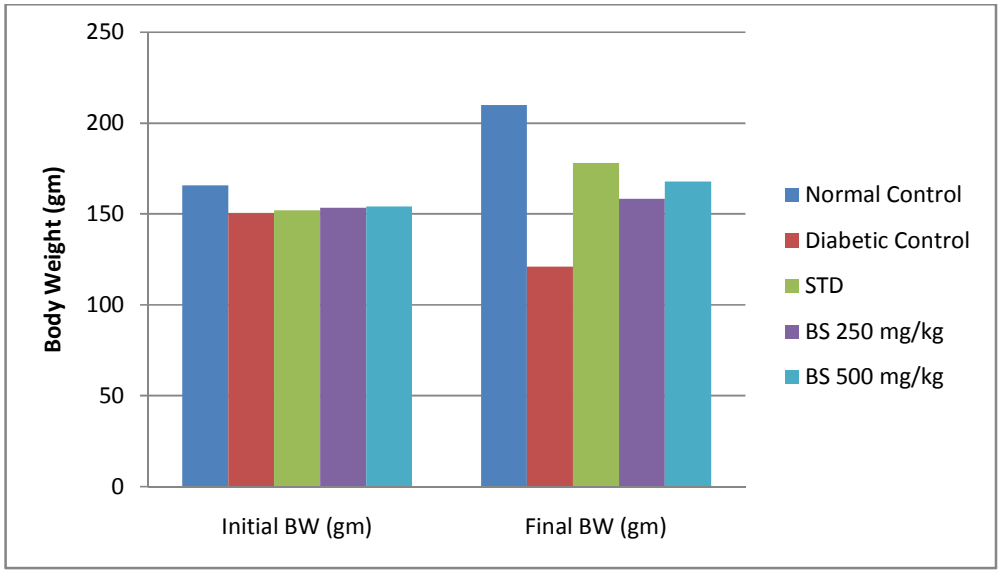

Fig. 2. Effect of ethanolic extract of BS on the body weight after 21 days feeding in alloxan induced type-2 diabetic rats

*[N.B: Data were analysed 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]*

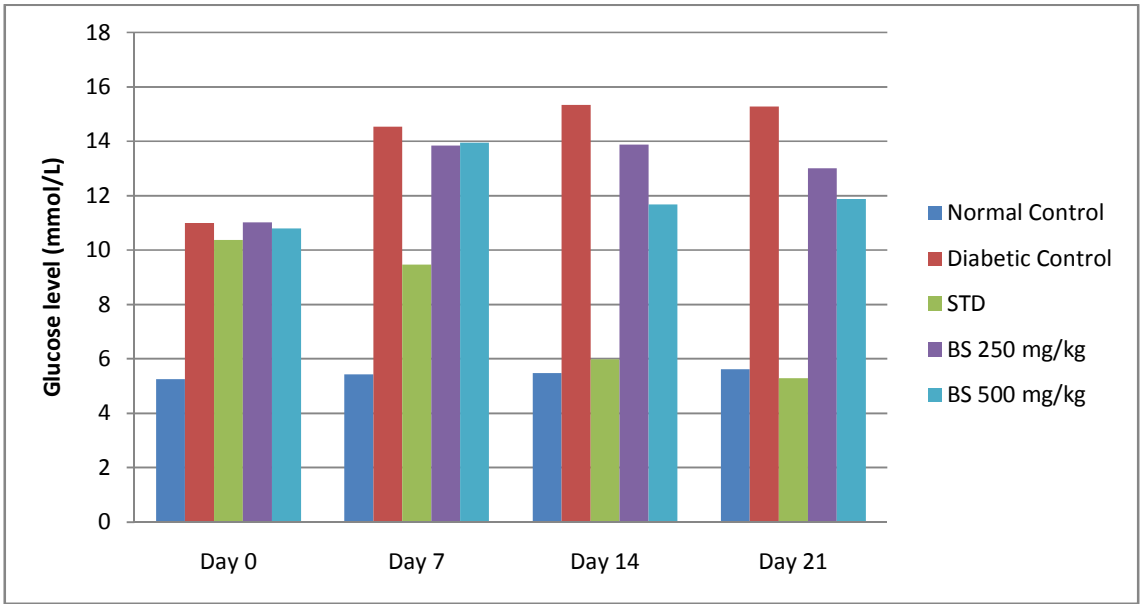

Fig. 3. Effect of ethanolic extract of BS on the fasting blood glucose level after 21 days feeding in type-2 diabetic rats

*[N.B: Data were analysed 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]*

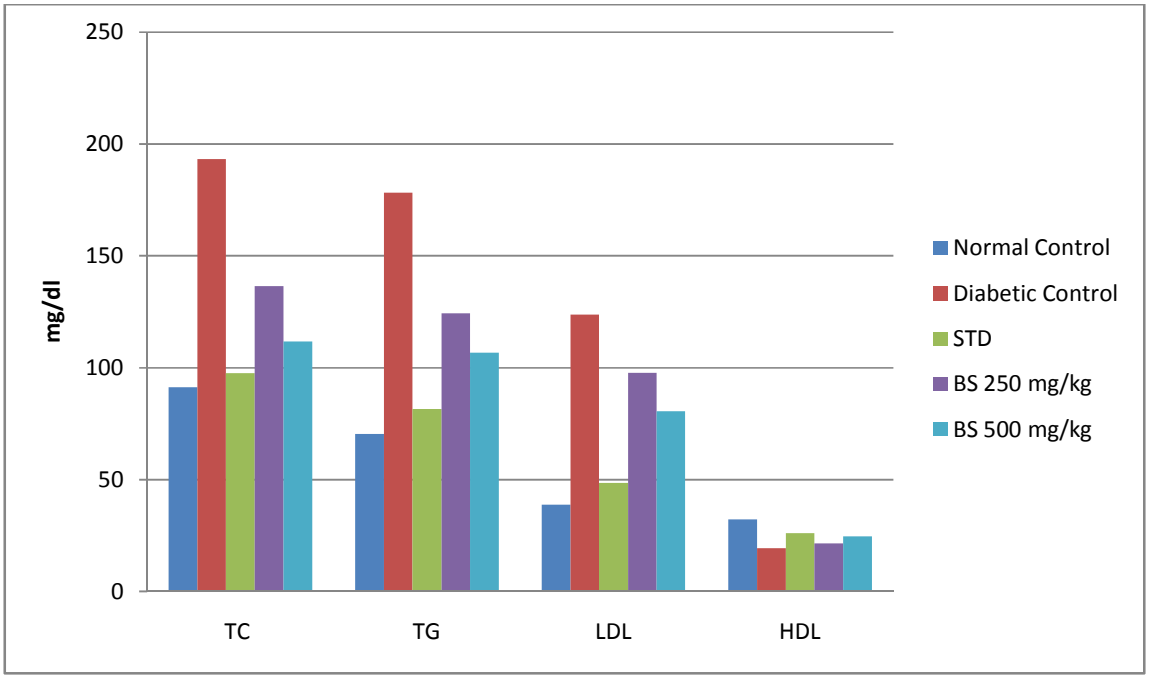

Fig. 4. Effect of BS on total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C level after 21 days feeding in type-2 diabetic rats

*[N.B: Data were analysed 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]*

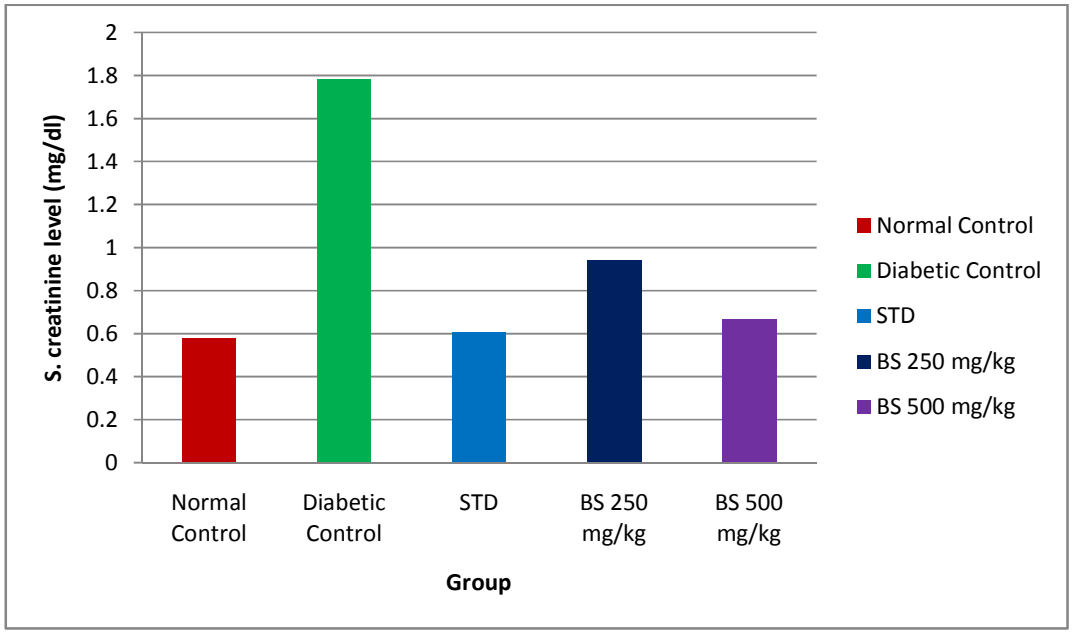

Fig. 5. Effect of BS extract on the serum creatinine level after 21 days feeding in type-2 diabetic rats

*[N.B: Data were analysed 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]*

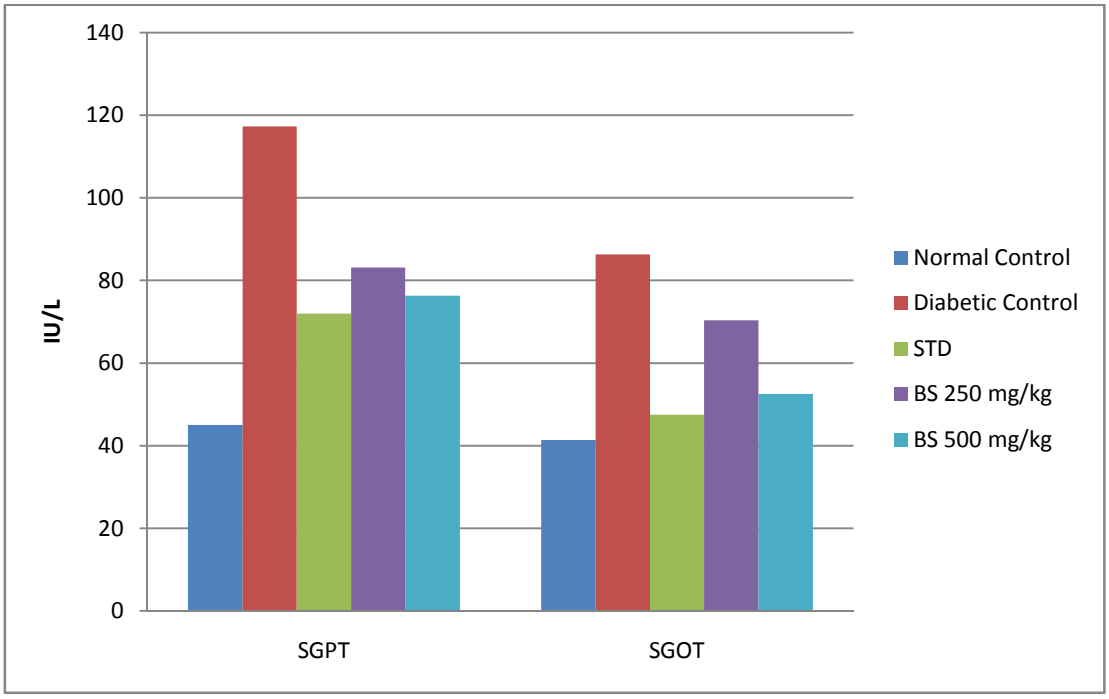

Fig. 6. Effect of BS extract on the SGPT and SGOT level after 21 days feeding in type-2 diabetic rats

*[N.B: Data were analysed 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]*

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

It is known that loss of body weight and decreased growth rate in diabetic rats is due to increased catabolism of protein and muscle wasting [27]. Rats treated with BS extract improved body weight suggesting that the higher dose of BS could be protective against protein degradation by improving glycemic control (Fig. 2).

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

After a 21 days chronic study, the BS extract at both 250mg and 500mg/kg dose on type 2 diabetic rats showed highly significant reduction in serum glucose level ($p<0.001$) whereas, glibenclamide (5mg/kg) showed very highly significant ($p<0.001$) reduction of glucose level. Therefore, the extract has comparable antidiabetic activity with glibenclamide (Fig. 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 [28]. 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 (Fig. 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 [29].

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 [30]. Serum creatinine level is a biomarker of renal function.

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

Increase in the plasma SGPT and SGOT are observed in the condition in which pancreas, liver, kidney and heart are destroyed [31]. The present results showed that injection of alloxan induces a hepatocellular damage, which is indicated by significant increase in SGPT and SGOT in diabetic group as compared to control group (Fig. 6).

In the present study, ethanolic extract of *B. stipularis* significantly decreased SGPT and SGOT enzyme level in diabetic rats. The improvements in the levels of the enzymes studied are a consequence of an improvement in the carbohydrate, fat and protein metabolism [32]. Therefore, BS treatment showed significant hepatoprotective activity in alloxan inuced type-2 diabetic rats.

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

4. CONCLUSION

The overall findings of this study focused that treatment of alloxan-induced diabetic rats, with the extract of *Bridelia stipularis* for 21days, could restore normal bioactivities by shifting lipid and carbohydrate metabolism homeostasis. Furthermore, BS showed significant nephroprotective and hepatoprotective actions.

Therefore, it can be concluded that alcoholic extract of this plant can be successfully utilised for the management of diabetes. In future, it is recommended to determine the mechanism(s) involved and, to identify the responsible active compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All protocols for animal experiment were approved by the institutional animal ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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