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Hypoglycemic Activity of *Bridelia Stipularis* on Combination of High Fat Diet and STZ Induced Diabetic Rats

Authors
Neethu R, Sugandha G Chaudhari, Vikram Bafna, Rajendra Patil
Mukesh Kumar Chavan, Geeta Basaiye
Dr. L.H. Hiranandani College of Pharmacy
Corresponding Author
Neethu R
Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar-3, Mumbai, Maharashtra, India
Email: *neethuraveendranath@gmail.com*

ABSTRACT

The aim of this study is to explore Bridelia stipularis which may lead to discovery of new therapeutic agents which may treat hyperglycemia and in future it may be used in diverse disease condition where hyperglycemia is the main source of amplifying and complication of other major disease processes. with the aim, the objectives of the study includes identification of active constituent which will be crucial in understanding the mechanism of action of the said activities and to evaluate the anti-diabetic activity of the Bridelia stipularis leaf extract in HFD and STZ induced diabetic models. Sprague–Dawley (SD) rats (180–200 g) of either sex were allocated for dietary regimen of water and in house prepared high fat diet for two weeks. The HFD-fed rats exhibited significant increase in body weight, basal plasma glucose (PGL), triglycerides (PTG) and total cholesterol (PTC) levels. After 2 weeks of dietary manipulation, rats were injected intraperitoneally with low dose of streptozotocin (STZ) (35 mg kg−1). In addition, the levels of PTG and PTC were further accentuated after STZ treatment in HFD-fed rats. Thus, these fat-fed/STZ-treated rats simulate natural disease progression and metabolic characteristics typical of individuals at increased risk of developing type 2 diabetes because of insulin resistance and obesity. The present study represents that the combination of HFD-fed and low-dose STZ-treated rat serves as an alternative animal model for type 2 diabetes and Bridelia stipularis can be used for treating hyper glycemia.

INTRODUCTION

Diabetes mellitus is one of the most common endocrine disorder characterised by hyperglycemia. The epidemic of type 2 diabetes is a major growing public health problem that threatens to reduce life expectancy and increase morbidity as a result of its complications.[1] .It is characterised by progressive decline in insulin production or action and followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction.)[2] Insulin resistance, defined as a state of reduced responsiveness to normal circulating levels of insulin plays an important role in the development of type 2 diabetes.[3] Hyperglycae-mia and cardinal feature of diabetes, can be controlled either by the exogenous administration of insulin or through oral anti diabetic drugs which increase insulin secretion.[1] .)According to report of International Diabetes Federation 2011, by the end of 2025 the number of diabetic patients will reach up to 300 million.[3]

Obesity and dietetic/sedentary life style plays an important role in the development of type II diabetes in this era.[2] The first transition is the shift from a healthy state to a prediabetes state. In prediabetes, patients have either impaired fasting glucose, impaired glucose tolerance, or both, and is often associated with insulin resistance. In healthy individuals, the adipose tissue functions as a safe storage site for lipids during a positive caloric balance. Likewise, excess circulating glucose is accommodated by the liver and muscle tissue in the form of glycogen. In the context of fully occupied glycogen stores, high glucose levels might also bring about de novo lipogenesis, occurring mainly in the liver and, to a lesser extent, in the adipose tissue. De novo lipogenesis helps maintain normal blood glucose levels by sequestering away excess glucose from the circulation. Normoglycemia in healthy individuals is maintained by the unique interplay between the almost opposing hormones, insulin and glucagon. The dialogue between these two hormones becomes perturbed with the disease progression of type 2 diabetes. The transition from a metabolically healthy state to pre diabetes often includes an obese state characterized by hyper insulinemia, insulin resistance, and dyslipidemia. However, it should be stressed that both metabolically healthy obese individuals, as well as metabolically unhealthy lean individuals, can be found in the general population.

This implies that obesity might not automatically or immediately result in the development of type 2 diabetes, and highlights that type 2 diabetes is a highly polygenic and hetero genous disease. The nutritional overload, which in the long term leads to obesity, can quickly induce insulin resistance in skeletal muscle as well as in the liver. Insulin resistance in skeletal muscle might reduce the occurrence of lipotoxic effects in muscle by redirecting the excess energy to the adipose tissue stores, and can thus be seen as a normal physiological function in healthy individuals. Severe expansion of the adipose tissue is tightly associated with adipose inflammation and a distorted adipokine profile,marked by high leptin and low adiponectin levels30 representing dysfunctional adipocytes. Dysfunctional adipose tissue leads to ectopic fat accumulation in non-adipose tissue, such as muscle, liver, and b-cells.

Intramyocellular lipid accumulation is associated with insulin resistance. Insulin-resistant muscles have lower glycogen synthesis and redirect glucose to the liver, where it contributes to hepatic lipid accumulationthrough de novo lipogenesis. Hepatic fat accumulation can induce hepatic insulin resistance, with decreased glycogen synthesis and increased gluconeogenesis. This impaired insulin-induced suppression of hepatic glucose output may contribute to hyperglycemia Further inflammation of the abdominal adipose tissue may worsen the dysfunctional state of the adipocytes, leading to more ectopic fat accumulation, insulin resistance and hyperinsulinemia, in a negative feedback loop[(4).]

In India, indigenous remedies have been used in the treatment of diabetes mellitus since the time of Charaka and Sushruta (6th century). The World Health Organization (WHO) has listed 21,000 plants which are used for medicinal purposes around the world. Among these, 2500 species are in India. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug.[(5)]

In ancient times plants and herbs were used as remedy for serious health complications. Herbal drugs have lesser or no side effects and are less expensive as compared to synthetic drugs. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world. Many indigenous Indian medicinal plants have been found to be useful for managing diabetes. After recommendation made by World Health Organization on medicinal plants for anti-diabetic drugs, many researchers focused on traditional medicinal plants for more effective and safer hypoglycemic agents. Many useful plants and herbs introduced in pharmacological and clinical trials have been confirmed their blood sugar lowering effect[(6)]

The plant kingdom has become a target for the search of biologically active lead compounds by multinational drug companies for the treatment of various diseases. Many medicinal plants become a part of our diet in form of fruits and vegetables. Potential source of many drugs used in modern medicine is from those medicinal plants. For example, quinine, opium alkaloids, atropine, cardiac glycosides (digitalis) and the popular hypoglycaemic drug glucophage (metformin), derived from *Galega officinalis*. Traditional anti-diabetic plants may provide new oral anti-diabetic compounds, which can counter the high cost and poor

Availability of the current medicines for many rural populations in developing countries[(7).]

Bridelia stipularis Blume is distributed in in the forest areas of the central and eastern parts of Bangladesh. It is also found in India and Myanmar and in India it is found in koorge districts of Karnataka and the warmer regions of India[(8).]Tannins and teraxenone were isolated from leaves and bark of the plant were supposed to be responsible for the medicinal properties of plant. Bark decoction is used in the traditional medicine for the treatment of asthma, intestinal worms and cough, while leaves are used against colic's.[(9)] The plant is used in the treatment of amoebic dysentery, chest pain, constipation, diarrhea, leucoderma and strangury Decoction of bark is used for cough, fever and asthma. It also

showed hypotensive and hypoglycaemic actions on animals. Leaves are used for jaundice.[8]

BOTANICAL DESCRIPTION[10]

Climbing Bridelia is a large, evergreen climbing shrub. Leaves are 5-20 cm, somewhat leathery, elliptic-obovate or orbicular-oblong. Flowers are small, in small axillary clusters or long spikes, often subtended by long stipular bracts. Flowers are borne in usually 2-6-flowered clusters in leaf axils, sometimes grouped into many-flowered spikes or on terminal small-leaved branches. Male flowers are 0.6-1 cm in diameter, on very short stalks up to 1 mm. Receptacle is cup-shaped; sepals ovate-triangular, about 4 x 2.5 mm. Petals are spoon-shaped, about 2 mm, 3-5-toothed, disk shallowly cup-shaped, 5-6 mm in diameter. Fruits are oblong, 1.2 cm long, sitting on an enlarged calyx.

CHEMICAL CONSTITUENTS

Bark contains friedelin and ß-sitosterol and tannins. Root contains teraxerone .Leaves contains tannins and fatty alcohol, named bridelyl alcohol besides fatty acids and a phlobatannin.[9]

2. MATERIALS AND METHOD

2.1. Materials

STZ was purchased from High media MUMBAI. The feed ingredients such as casein and cholesterol (both from Himedia laboratories, Mumbai, India. dl-methionine vitamin and mineral mix and yeast powder were procured from Molychem, MUMBAI. Metformine was purchased from high media MUMBAI.

2.2. Plant materials

The leaves of plant Bridelia stipularis were collected from koorge district of Karnataka. Plant was authenticated from Khalsa College of Arts Commerce And Science, Mumbai (specimen #: nr 0211013).

2.3 Extraction of plant material

Plant extract were prepared by soxlet extraction procedure. Before that plant material was socked in chloroform in order to remove chlorophyll and other fatty substances from it. 100gm of dried powder was socked in 200 ml of ethanol and kept for soxlet extraction for 48 hrs. The extract so obtained was filtered through vacuum fllter. so obtained filtrate was dried in rotary evaporator until a constant dry weight of extract was obtained. The final crude extract was stored at room temperature.

2.4 Animals

Male Sprague–Dawley (SD) rats (160–180 g) were procured from from Bharath serum limited.. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22±2 ◦C) and humidity (55±5%). The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

2.4 **Acute toxicity studies**

Sprague–Dawley (SD) rats (180–200 g) of either sex were procured from Bharath serum limited.

The animals were allocated for dietary regimen of water and in house prepared high fat diet .The each animal received a single oral dose (2000 mg/kg, body weight) of each fraction. Animals were kept overnight fasting prior to drug administration. After the administration of the fraction, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention during the first 4 hours) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, autonomic (salivation, lacrimation, perspiration, pilo-erection, urinary incontinence and defecation) and CNS (ptosis, drowsiness, gait, tremors and convulsions) changes (OECD, 425).

2.5 Selection of dose:

Based on stabilization studies, the doses of the plant extract were taken as 200mg/kg, 300mg/kg, 400mg/kg. whereas dose of standard drug was taken as 180mg/kg.

2.6 Experimental design[10,11,12]

Firstly, stabilization studies were conducted on 6 animals for deducing the dose of STZ to be given. From the stabilization studies, a low dose of stz was confirmed which was 35mg/kg of body weight with combination of high fat diet.

Before induction of the disease, the animals were fasted overnight. The next day, fasting blood sugar levels were obtained using the glucometer. The rats were given an intraperitoneal injection of streptozotocin (35 mg\kg) freshly prepared in 0.1M sodium citrate buffer. The time taken for the disease to be induced ranges between 2-7 days. After 7 days, blood glucose levels were checked. Blood glucose levels above 300 mg/dl confirmed diabetes. Then the animals were divided into 6 groups having 6 animals in each group. The groups are as follows. Rats were divided in to six groups each consists of six animals. Group I –as Non diabetic control. Group II –as Diabetic Control and Group III as standard (metformin) Group IV, GroupV, group VI were administered with three different doses (200mg/kg.300mg/kg, 400mg/kg) of plant extract respectively.

2.7 Estimation of biochemical parameters

Blood glucose levels. Serum triglyceride level and Serum cholesterol levels were estimated for biochemical parameters.

2.8 Histopathological studies

Histopathological investigation was carried out after completion of treatment. Both control and experimental rats were scarified, liver tissues were isolated. On glass slides 10% formalin was fixed over the sliced piece of liver and tissues for 3 d and observed under microscope.

2.9 Statistical analysis

The values were expressed in mean ± standard deviation. The statistical analysis was carried out by using One-way ANOVA in standard statistical software package of social science (SPSS).

RESULTS

In acute toxicity studies of *Bridelia stipularis* by oral route did not showed any mortality and toxic effects up to the dose of 2000mg/kg body weight. According to those other safe doses of plant extract was selected for further experiments.

STZ- induced diabetic rats exhibiting persistent hyperglycemia (Blood Glucose>300 mg\dl) were selected for assessing the effect of BS. To observe long term effect, sub acute study of 28 days was done. Table 1 shows the results of this study. BS (400mg/kg) showed highly significant ($p<0.001$) hypoglycaemic effect when compared to diabetic control. while BS (300mg/kg) and 200mg/kg) also showed less significant ($P<0.01$) hypoglycaemic effect when compared with disease control. In sub acute study BS (400mg/kg) is more effective than BS (200mg/kg).

STZ induced diabetic animals were assessed by lipid profile. Oral administration of ethanolic leaf extract of BS of different doses such as (400mg/kg/300mg/kg/200mg/kg) was carried out in experimental animals. Results revealed that the levels of glucose, triglycerides and total cholesterol were significantly increased in STZ-induced diabetic animals when compared to that of normal animals. Table 2 shows results of this study. STZ-induced diabetic animal showed increase in hyperglycemia, were significantly depleted when compared to that of control rats. After supplementation of ethanolic leaf extract of BS at the dosage of 400 mg/kg in the STZ induced diabetic rats, a significant reduction in glucose, triglycerides, total cholesterol were recorded when compared to normal animals.

Table: 1 Effect of BS on Blood Sugar (BSL) in STZ induced rats.

GROUPS	0^{th} day	7^{th} day	14^{th} day	21^{st} day	28^{th} day
Control	93±3.347	93.33±1.498	93.33±1.478	93±1.498	93±1.366
Diseasecontrol	456±6.085	522±14.24	514±10.80	512±14.24	456±6.085
Standard	465±70463	430±5.388	365.0±3.651	279±1.537***	104.7±1.256***
BS (200mg/kg)	465±2.950	420.7±2.963	388.0±6.429	290.7±5.239**	132.7±1.256**
BS (300mg/kg)	467±3.856	433.3±3.575	382.5±3.099	273.0±3.367**	125.7±1.80***
BS (400mg/kg)	452±6.314	403±5.774	359.2±7.683	250.0±4.282***	115.5±2.460***

All values are expressed as mean ± S.E.M (n=5). *P<0.05, **P<0.001 as compared to diabetic control. One-way ANOVA followed by Dunnet multiple comparison test.

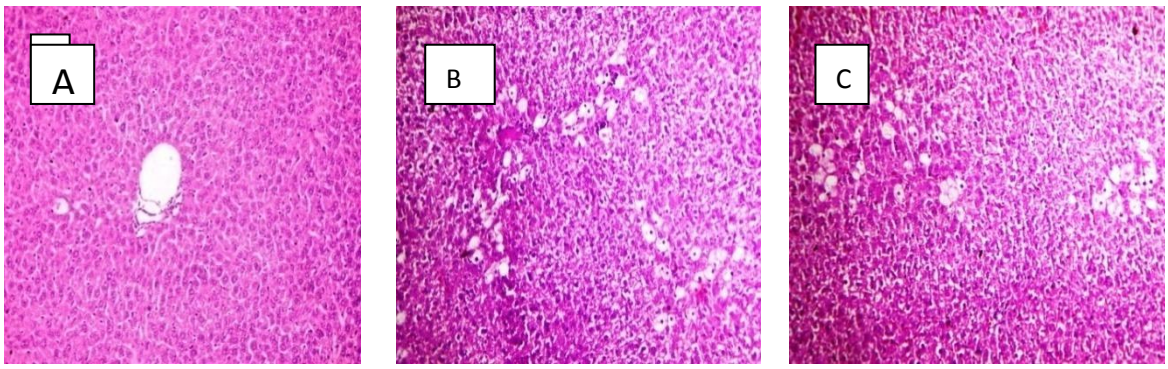
Table 2: Effect of BS on serum triglyceride and cholesterol in STZ induced rats.

GROUP	TRIGLYCERIDE		CHOLESTEROL	
	0 DAY	28 th DAY	0 DAY	28th DAY
Control	129.2±3.005	139.3±7.519	105.2±1.447	105.2±1.447
Disease control	242.3± 3.993	170.3±1.321	207.4±4.353	231. ±3.940
Standard	237.6±7.346	139.7±2.393***	230.4±1.877	110.7±1.820***
BS (200mg/kg)	230.6±2.295	150.8±2.845**	233.2± 1.408	144.9±1.418**
BS (300mg/kg)	231.4±1.504	121.6±3.720***	232.2± 1.540	133.3±1.633***
BS (400mg/kg)	233.7±2.373	116.9±2.245***	234.5± 0.875	120±0.9039**

All values are expressed as mean ± S.E.M (n=5). *P<0.05, **P<0.001 as compared to diabetic control. One-way ANOVA followed by Dunnet multiple comparison test.

The histological studies were carried out to prove the efficacy and protective effect of o plant extract. Figure 1A shows a normal liver with no abnormalities and normal cell structure. In STZ-induced diabetic rat congestion of portal vessel, and hemorrhagic necrosis of the liver cells were observed (Figure 1B). The liver cells of ethanolic leaf extract of BS fed rat revealed restoration of the hepatic tissue architecture (Figure 1E AND 1F). In the Group-1C treated rat, the liver cells showed a normal lobular architecture and that is of standard drug metformine.

Figure 1: Histopathological studies of liver tissue of diabetic rats treated with ethanolic extract of *Bridelia stipularis* for 28 days.



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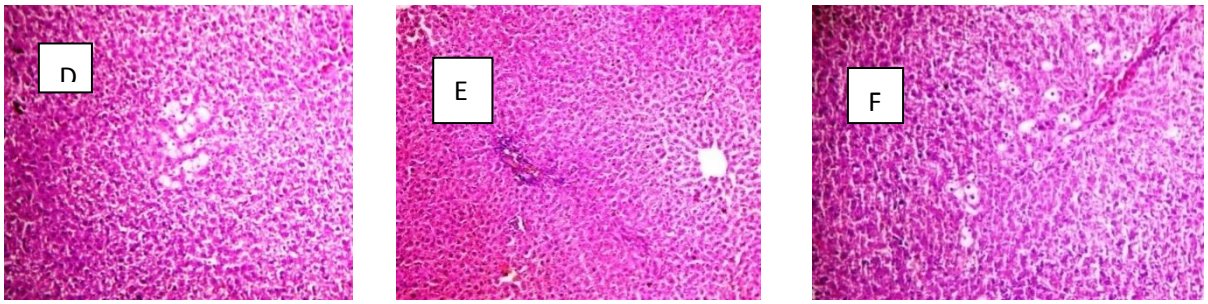
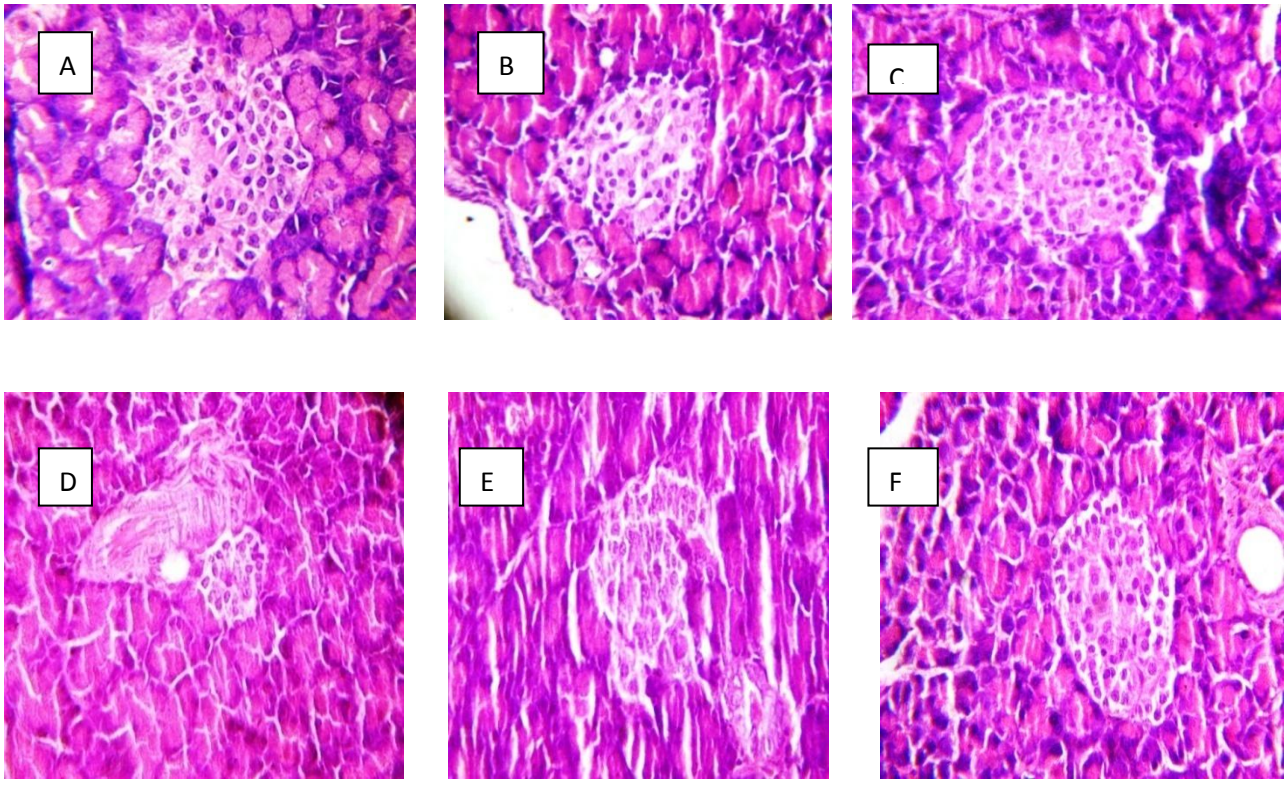


A:Control,B:STZ Induced Diabetic Rats, C:Rats Treated With Standard Drug Metformine, D: BS (200mg/kg) ,E:BS (300mg/kg),f:BS (400mg/kg).

Figure 2: Histopathological studies of pancreatic tissue of diabetic rats treated with ethanolic extract of *Bridelia stipularis* for 28 days

In STZ-induced diabetic rat there was moderate degenerative change (++) of the cells of islets of the langerhans (Endocrine pancreas) with depletion of the cells of the Islet (Figure 2B). The pancreatic cells of ethanolic leaf extract of BS(300mg/kgand 200mg/kg) fed rat revealed mild to moderate degenerative change(+)of the cells of islets of the Langerhans (Figure 2D AND 2E). In the Group-2C treated rat, the pancreatic cells showed a normal cells of islets of langerhans and that is of standard drug metformine .The dose of BS (400mg/kg) showed no any abnormalities as like control rats.(Figure 2F).

Figure 4: Histopathological studies of pancreas tissue of diabetic rats treated with ethanolic extract of *Bridelia stipularis* for 28 day



A:Control,B:STZ Induced Diabetic Rats ,C:Rats Treated With Standard Drug Metformine ,D: BS (200mg/kg) ,E:BS (300mg/kg),F:BS (400mg/kg).

DISCUSSION

This study was initiated with the objective of developing an ideal model for type 2 diabetes that would closely reflect the natural history and metabolic characteristics of human type 2 diabetes and respond to the pharmacological treatments. Thus initial attempt was directed towards finding the threshold dose of STZ ie low enough to guarantee the development of type 2 diabetes in HFD rats. The dose of STZ (35 mg kg−1, i.p.) that produced frank hyperglycemia in HFD fed rtas. The HFD rat model with low dose of STZ (35 mg kg−1) thus can be more considered to represent the pathophysiological state of type 2 diabetes and was accompanied by marginal increase in body weight in contrast to the catabolic loss of body weight.

Briefly, the presence of high level of triglycerides due to excess fat intake could constitute a source of increased fatty acid availability and oxidation. The preferential use of increased fatty acids for oxidation blunts the insulin-mediated reduction of hepatic glucose output and reduces the glucose uptake or utilization in skeletal muscle leading to compensatory hyperinsulinemia, a common feature of insulin resistance.

In the present study, both extracts significantly reduced the triglyceride levels in treated diabetic rats when compared to untreated diabetic rats. The ethanolic leaf extract was also able to significantly deplete the total cholesterol concentration in treated STZ-induced diabetic rats. These reductions could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics.

The histopathological studies of diabetic rats showed necrosis of the hepatic cells, degeneration, vacuolation in hepatic cells in comparison to that of control. Similar observations were observed during STZ-induced diabetic rat .These damages may be due to oxygen free radicals exerting their cytotoxic effect by peroxidation of membrane phospholipids leading to a change in permeability and loss of membrane integrity. After supplemented with ethanolic leaf extract of Bridelia stipularis may reduce membrane integrity.

In the present study, it can be concluded that Bridelia stipularis has significance hypoglycaemic activity.

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