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Antidiabetic Activity of Methanolic Extract of *Hiptage bengalensis* Leaves in Alloxan Induced Diabetic Models

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Abstract: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and its occurrence is increasing fast in most of the countries. Herbal medicine derived from plant extracts have been utilized increasingly for the treatment of various disorders like diabetes mellitus. The present study was designed to evaluate the antidiabetic activity of Methanolic Extract of *Hiptage bengalensis* L. Kurz (MEHB) in alloxan induced diabetic rats and chick model. Alloxan (120 mg kg^{-1}) was used to induce diabetes in rats and the blood glucose levels were estimated by using commercial kit in the market. The methanolic extract of *Hiptage bengalensis* was administered to diabetic rats as single dose for one day at a dose of 100 and 200 mg kg^{-1}. The extract produced a significant reduction ($p<0.01$) of blood glucose levels at a dose of 100 and 200 mg kg^{-1} in diabetic rats. It also showed a beneficial effect on the lipid profile in alloxan induced diabetic rats. These results showed that methanolic extract of *Hiptage bengalensis* produced a dose dependant antihyperglycemic activity in rats.

Key words: Diabetes mellitus, antihyperglycemic, glibenclamide, *Hiptage bengalensis,* alloxan

INTRODUCTION

Diabetes mellitus is one of the most common metabolic disorder with long term macrovascular and microvascular complications includes diabetic nephropathy, neuropathy and retinopathy that results in significant morbidity and mortality (Heath *et al*., 2009). This metabolic disorder is seen world wide and its occurrence is increasing fast in most of the countries (Siddharth, 2001). Diabetes mellitus is a chronic metabolic syndrome characterized by hyperglycaemia (Kesavulu *et al*., 2000). Treatment for diabetes mellitus includes insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguamides (Tiwari and Madhusudana Rao, 2002).

Herbal medicine derived from plant extracts have been utilized increasingly for the treatment of various disorders like diabetes mellitus, liver diseases (Chattopadhyay, 2003), atherosclerosis, acute hypertension with little known mechanism of action (Jeong *et al*., 2002).

Hiptage bengalensis (L.) Kurz (synonym: *Banisteria bengalensis* and *Hipatage madablota*) is a herb and is belongs to the family of Malpighiaceae. It is distributed throughout India, Srilanka and Andaman Islands, Bangladesh and Myanmar to southern China (Parotta, 2001). The plant is large, evergreen, climbing shrub with brownish bark peeling off in flakes; young parts silky. Leaves opposite, coriaceolate to elliptic-oblong, apex acute to acuminate, base rounded to cuneate and the margins are entire. In India, flowering occurs mainly between February and April, sometimes also in October; fruiting occurs mainly from April to June. In ayurveda the leaves and bark are considered vulnerary; the leaves are highly regarded for treating skin diseases. (Bhukya *et al*., 2009) have evaluated that leaves exhibited analgesic and anti-inflammatory activity. The leaf juice possesses insecticidal properties and is used an external application for scabies. The leaves and bark of *Hiptage bengalensis* showed a significant antifungal activity (Bobbarala *et al*., 2009). Leaves also possess antioxidant activity (Amudha and Shanthi, 2011). The plant is also used in the treatment of chronic rheumatism and asthma (Parotta, 2001). The present study has been undertaken to evaluate the antidiabetic activity of the methanolic extract of *Hiptage bengalensis* (*H. bengalensis*) in alloxan induced diabetic rats.

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MATERIALS AND METHODS

Plant material: The leaves of *H. bengalensis* were collected from Thirupathi Hills Andhra Pradesh, India in November 2010. It was authenticated by Prof. V. Raju, Department of Botany, Kakatiya University and Warangal, India.

Preparation of extract: The leaves of *H. bengalensis* were dried in shade at room temperature. The dried leaves were coarsely powdered and it is macerated with methanol. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure at 50-55°C and stored in vacuum desiccator. The suspension of the extract was prepared using 0.5% sodium Carboxy Methyl Cellulose (CMC) and was used in the entire experimental studies.

Phytochemical screening: The methanolic extract of *Hiptage bengalensis* was screened by using standard methods for the presence of various phyto-constituents like steroids, alkaloids, terpenoids, glycosides, flavonoids and carbohydrates (Kokate, 1994; Harborne, 1998).

Chemicals: Alloxan monohydrate (Sigma, St Louis, USA), Glibenclamide (gift sample, Dr. Redyy's, Hyderabad), Methanol (E-Merck, Mumbai, India), Sod CMC (Central Drug House, New Delhi).

Animals: Male albino Wistar rats weighing 150-180 g (4-8 weeks) used in this study were procured from Mahaveera Enterprises, Hyderabad and were housed in polypropylene cages in a room of temperature 23±2°C and relative humidity 50% with 12:12 h light:dark cycle. Animals were acclimatized to this environment throughout the period of experimental study. They were with standard food and water *ad libitum*.

Acute toxicity study: Acute toxicity studies were performed by using male Wistar rats. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. MEHB was administered orally using various doses upto 2000 mg kg^{-1} and observed for the mortality and behavioral changes (OECD, 2000).

Evaluation of anti-diabetic activity in rat model: Diabetes mellitus was induced in rats by administration of alloxan monohydrate (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) at dose of 120 mg kg^{-1} intraperitoneally in normal saline (Ragavan and Krishnakumari, 2006). After 1 h of alloxan administration the animals were given feed *ad libitum* and 5% dextrose solution were also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation for about 48 h. The animals were kept fasting overnight and blood glucose levels were estimated before and after 72 h of alloxan treatment. Animals showing blood glucose levels of >200 mg dL^{-1} is considered as diabetic and were used for study.

Study design: All the fasting (for 12 h) diabetic rats were divided into four groups of each six (n = 6):

Group 1: Normal rats served as normal control and treated with (1 mL kg^{-1}, p.o.) of 0.5% Sod CMC
Group 2: Served as diabetic/disease control and received 0.5% Sod CMC (1 mL kg^{-1}, p.o.)
Group 3: Diabetic Rats treated with MEHB at a dose of 100 mg kg^{-1}, (p.o.)
Group 4: Diabetic Rats treated with MEHB at a dose of 200 mg kg^{-1}, (p.o.)
Group 5: Diabetic rats treated with glibenclamide (2.5 mg kg^{-1}, p.o.) and served as standard group (Dash *et al*., 2008)

The treatment was given for 1 day and blood samples were collected at different intervals.

Collection of blood samples: Blood samples were collected from all the groups of animals at 0, 1, 3, 5, 7 h intervals through puncture of retro orbital plexus and were centrifuged at 10000 rpm for 10 min. Serum was separated and stored at -20°C and then used for estimating blood glucose (Span Diagnostics, Surat, India), Triglyceride, HDL-Cholesterol, Total cholesterol levels (Excel Diagnostics, Hyderabad).

Evaluation of anti-diabetic activity in chick model

Induction of diabetes in chicks: Fertile eggs of (30/60 g) country chicken were obtained and incubated for 14 days (99°F 0.87% humidity) in a suitable incubator. On the 14th day of incubation, a small holes were made on the shells using driller and then the toxic drug alloxan at a dose of 0.9 mg egg^{-1} was injected into the each egg under sterile conditions. A control group of 6 eggs were maintained. After alloxan/vehicle injection, holes were closed using plaster or tape, then eggs were incubated for another 7 days. On the 21st day chicks come out from eggs. After few days, glucose levels were estimated by taking the blood from tip of finger using chem strip method (Yoshiyama *et al*., 2005). Blood glucose levels were found as greater than 300 mg dL^{-1} were considered as hyperglycemic when compared with the normal levels (230.8±13.9). This indicated the successful induction of diabetes in chicks.

Study design: All the fasting (for 12 h) diabetic chicks were divided into four groups with each group with six animals (n = 6):

Group 1: Chicks served as normal control and treated with (1 mL kg^{-1}, p.o.) of 0.5% Sod. CMC
Group 2: Served as diabetic/disease control and received 0.5% Sod. CMC (1 mL kg^{-1}, p.o.)
Group 3: Diabetic chicks treated with MEHB at a dose of 100 mg kg^{-1}, (p.o.)
Group 4: Diabetic chicks treated with MEHB at a dose of 200 mg kg^{-1}, (p.o.)
Group 5: Diabetic chicks treated with standard, glibenclamide (2.5 mg kg^{-1}, p.o.) (Dash *et al*., 2008)

Blood samples were collected at 0, 1, 3 h of test drug administration and analyzed for blood glucose levels using strip method.

***In vitro* glucose uptake method using rat hemidiaphragm:** *In vitro* glucose uptake by the rat hemidiaphragm models has been used to assess the *in vitro* antidiabetic activity of various plant extracts (Chattopadhyay *et al*., 1992). In the present study, *in vitro* glucose uptake by rat hemidiaphragm method to evaluate the antidiabetic activity of methanolic extract of HB. The selected rats were killed by decapitation and diaphragms were taken out quickly avoiding trauma and divided into two halves. The hemidiaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and were placed in a culture tubes containing 2 mL tyrode solution with 2% glucose and incubated for 3 h at 37°C in an atmosphere of 100% O_2 with shaking at 140 cycles min^{-1}.

Study design: Four sets of experiments were performed. The animals were killed by decapitation and diaphragms were exposed to:

Group 1: Two mililitter of tyrode solution with glucose (2%) only and served as control
Group 2: Two mililitter of tyrode solution with glucose (2%)+0.5 mL of MEHB (100 mg mL^{-1})
Group 3: Two mililitter of tyrode solution with glucose (2%)+0.5 mL of MEHB (200 mg mL^{-1})
Group 4: Two mililitter of tyrode solution with glucose (2%) +/0.62 mL of insulin (0.4 Unit mL^{-1})
Group 5: Two mililitter tyrode solution with glucose (2%)+/0.62 mL of insulin (0.4 Unit mL^{-1})+0.5 mL of MEHB (200 mg mL^{-1}) (Walaas and Walaas, 1952; Chattopadhyay *et al*., 1992)

Statistical analysis: All the experimental values were expressed as Mean±SD (n = 6). One-way ANOVA and Dunnett's test were used to compare means from the control group and each of the groups exposed to toxicant and MEHB the statistical significance was judged at the 0.05 probability level.

RESULTS

Phytochemical screening: Preliminary phytochemical screening of the Methanolic Extract of *Hiptage bengalensis* (MEHB) results showed the presence of steroids, terpenoids, carbohydrates, phenolics and glycosides.

Acute toxicity study: No adverse effects and mortality of the animals were observed during the period of the study, 24 h up to the dose of 2000 mg kg^{-1} body weight p.o. of methanolic extract of HB.

Effect of MEHB on blood glucose levels in alloxan induced diabetic rats: The effect of MEHB on blood glucose levels were shown in Table 1. Alloxan monohydrate administration at a dose of 120 mg kg^{-1} to rats successfully produced diabetes by elevating blood glucose levels grater than 200 mg dL^{-1}. Administration of MEHB at a doses of 100 and 200 mg kg^{-1} to diabetes rats produced a significant reduction in the blood glucose levels in a dose dependant manner ($p<0.01$). The significant glucose levels were reduced after 1 h of administration of MEHB. The maximum reduction of blood glucose levels were observed at 3rd h i.e., 29.4 and 35.1% with doses of 100 and 200 mg kg^{-1}, respectively (Table 1). However, the antidiabetic activity of MEHB of 200 mg kg^{-1} was comparable with known standard drug like glibenclamide.

Table 1: Effect of methanolic extract of *Hiptage bengalensis* (MEHB) on serum glucose levels in diabetic rats

Group/time	0 (h)	1 (h)	3 (h)	5 (h)	7 (h)
Normal control	89.9±5.60	91.2±7.80 (1.44)	90.2±8.40 (0.33)	88.8±7.50 (1.22)	90.5±8.70 (0.66)
Diabetic control	302.3±22.8	306.8±27.6 (1.48)	315.2±30.4 (4.26)	348.9±21.7 (15.4)	303.0±23.6 (0.23)
Diabetic+MEHB (100 mg kg^{-1})	271.6±20.7	246.2±22.5* (9.35)	191.6±17.6** (29.4)	195.2±14.9** (28.1)	200.0±14.2** (26.3)
Diabetic+MEHB (200 mg kg^{-1})	298.5±21.7	249.2±20.9** (24.8)	193.6±16.6** (35.1)	196.2±13.7** (34.2)	204.7±17.5** (31.4)
Diabetic+glibenclamide (2.5 mg kg^{-1})	272.9±20.5	226.3±19.6** (17.0)	187.9±16.1** (31.0)	174.1±13.9** (36.2)	175.6±13.3** (35.6)

All the values of Mean±SD, n = 6, *,**$p<0.001$ vs. diabetic control, in brackets % reduction of Glucose levels were mentioned

Effect of MEHB on lipid profiles in alloxan induced diabetes rats: Administration of Alloxan monohydrate elevated the serum lipid levels like Total Cholesterol (TC), Triglycerides (TG) and there is a decrease in High Density Lipoprotein (HDL-C) levels. Similar to the glibenclamide, MEHB at doses of 100 and 200 mg kg^{-1} to diabetic rats significantly ($p<0.05$) reduced the serum cholesterol levels after 3 h of its administration. The reduction of total cholesterol in rats was observed in dose dependant manner ($p<0.01$) (Table 2). The maximum reduction of total cholesterol was found at 5th h i.e., 18.32 and 24.32% at doses of 100 and 200 mg kg^{-1}. It also resulted in a significant ($p<0.05$) (Table 3) reduction in the serum triglycerides levels after 3h of its administration in a dose dependant manner. The maximum reduction of serum triglycerides in rats was observed at 5th h were 22.34 and 30.58% with 100 and 200 mg kg^{-1} ($p<0.01$) (Table 3). Administration of single dose of MEHB at rate of 100 and 200 mg kg^{-1} to diabetic rats significantly ($p<0.05$) (Table 4) increased the serum HDL-C levels after 3 h of its administration. The maximum increase in HDL-C levels was observed at 5th h were 60.0 and 74.2% with the doses of 100 and 200 mg kg^{-1}.

Effect of MEHB on blood glucose levels in alloxan induced diabetic chicks: Administration of MEHB at a doses of 100 and 200 mg kg^{-1} to diabetes chicks produced a significant reduction in the blood glucose levels in a dose dependant manner ($p<0.01$). The significant glucose levels were reduced ($p<0.01$) after 1 h of administration of MEHB. The maximum reduction of blood glucose levels were observed at 5th h i.e., 33.30 and 44.51% with doses of 100 and 200 mg kg^{-1}, respectively (Table 5). However, the antidiabetic activity of MEHB of 200 mg kg^{-1} was comparable with known standard drug like glibenclamide (Table 5).

Effect of MEHB on blood glucose levels (mg dL^{-1}) in rat hemi diaphragm method: Similar to the Insulin, in presence of MEHB at doses of 100 and 200 mg kg^{-1}, it was observed that there is enhancement of the glucose uptake by the hemi diaphragm, (skeletal muscle). The statistically significant difference ($p<0.01$) was observed in the utilization of glucose uptake in presence of MEHB by the hemi diaphragm and was observed as time dependant. The % of increase in glucose uptake by skeletal muscle by MEHB was 4.9 and 11.44% with

Table 2: Effect of methanolic extract of *Hiptage bengalensis* (MEHB) on lipid profiles (total cholesterol) levels in alloxan-induced diabetic rats

Group/time	0 (h)	1 (h)	3 (h)	5 (h)	7 (h)
Normal control	99.6±7.30	101.2±8.6 (1.60)	99.1±8.1 (0.50)	100.5±8.3 (0.90)	103.4±9.0 (3.81)
Diabetic control	139.8±12.1	141.2±12.0 (1.0)	142.7±12.6 (2.07)	140.6±11.8 (0.57)	142.1±11.0 (1.64)
Diabetic+MEHB(100 mg kg^{-1})	133.7±9.90	128.2±10.8* (4.11)	122.6±9.6* (8.30)	109.2±7.7** (18.32)	107.5±8.6** (19.59)
Diabetic+MEHB (200 mg kg^{-1})	136.1±10.8	130.2±11.9** (4.33)	110.6±9.5** (18.73)	103.0±9.6** (24.32)	102.1±9.0** (24.98)
Diabetic+glibenclamide (2.5 mg kg^{-1})	139.5±10.8	125.1±10.5** (10.32)	101.5±9.0** (27.24)	101.4±6.2** (27.31)	102.6±7.9** (26.45)

All the values of Mean±SD, n = 6, *,**p<0.05, 0.01 vs. diabetic control, in brackets % reduction of TC levels were mentioned

Table 3: Effect of methanolic extract of *Hiptage bengalensis* (MEHB) on lipid profiles triglycerides) levels (mg dL^{-1}) in diabetic rats

Group/time	0 (h)	1 (h)	3 (h)	5 (h)	7 (h)
Normal control	79.9±6.6	78.2±6.8 (2.12)	79.2±7.4 (0.84)	78.8±7.1 (1.37)	80.9±6.7 (1.25)
Diabetic control	137.0±10.9	139.2±11.8 (1.60)	144.7±11.7 (5.62)	147.6±12.2 (7.73)	145.4±13.6 (6.13)
Diabetic+MEHB (100 mg kg^{-1})	131.6±10.9	124.2±10.8* (5.62)	112.6±10.3** (14.43)	102.2±8.9** (22.34)	104.0±7.2**(20.97)
Diabetic+MEHB(200 mg kg^{-1})	138.3±11.6	124.2±10.3**(10.19)	110.1±10.1**(20.39)	96.0±8.4**(30.58)	97.0±9.3**(29.86)
Diabetic+glibenclamide(2.5 mg kg^{-1})	141.5±10.2	123.1±11.2**(13.0)	103.1±9.1**(27.13)	84.1±6.9** (40.56)	85.6±7.3**(39.50)

All the values of Mean±SD, n = 6, *,**p<0.01 vs. diabetic control, in brackets % reduction of TG levels were mentioned

Table 4: Effect of methanolic extract of *Hiptage bengalensis* (MEHB) on lipid profiles (HDL-C) levels (mg dL^{-1}) in diabetic rats

Group/time	0 (h)	1 (h)	3 (h)	5 (h)	7 (h)
Normal control	45.7±4.5	45.5±4.1 (0.43)	44.6±5.1 (2.40)	47.3±5.6 (3.50)	46.0±5.0 (0.65)
Diabetic control	24.8±2.1	25.0±1.9 (0.80)	25.6±3.1 (3.22)	24.5±2.9 (1.20)	25.1±2.1 (1.20)
Diabetic+MEHB (100 mg kg^{-1})	22.3±1.7	23.6±2.2 (5.82)	25.9±2.0* (16.14)	35.7±2.6** (60.0)	34.9±3.1** (56.50)
Diabetic+MEHB (200 mg kg^{-1})	22.5±2.1	24.8±1.9 (10.22)	29.3±2.4* (30.22)	39.2±2.8** (74.22)	37.9±3.2** (68.44)
Diabetic+glibenclamide(2.5 mg kg^{-1})	23.5±2.2	26.4±1.8 (12.34)	35.2±3.0** (49.78)	42.8±3.1** (82.12)	43.3±4.1** (84.25)

All the values of Mean±SD, n = 6, *,**p<0.05, 0.01 vs. diabetic control, in brackets % increase of HDL-C levels were mentioned

Table 5: Mean±SD of blood glucose levels (mg dL^{-1}) after administration of methanolic extract of *Hiptage bengalensis* (MEHB) in chick model

Group/time	0 (h)	1 (h)	3 (h)	5 (h)
Normal control	230.8±13.9	235.2±18.6 (1.90)	239.1±17.8 (3.59)	242.5±20.2(5.06)
Diabetic control	438.5±22.5	441.9±22.3 (0.77)	449.0±32.6 (2.39)	445.6±21.8(16.19)
Diabetic+MEHB (100 mg kg^{-1})	433.7±29.1	378.7±20.0**(12.68)	302.6±19.9** (30.22)	289.0±17.5** (33.3)
Diabetic+MEHB (200 mg kg^{-1})	456.0±30.8	350.2±21.9** (23.2)	280.6±19.2** (38.46)	253.0±17.6** (44.51)
Diabetic+glibenclamide(2.5 mg kg^{-1})	469.5±28.6	325.1±20.9** (30.75)	241.5±15.7** (48.56)	201.4±16.0** (57.10)

All the values of Mean±SD, n = 6, **p<0.01 vs. diabetic control, in brackets % reduction of glucose levels were mentioned

Table 6: Mean±SD of blood glucose levels (mg dL^{-1}) in rat hemi-diaphragm method

Group/time	0 (h)	1 (h)	3 (h)
Normal control	660.8±6.9	655.2±8.1 (0.84)	621.1±7.8 (6.0)
MEHB (100 mg mL^{-1})	633.7±9.1	623.7±7.0* (1.57)	602.6±9.9** (4.90)
MEHB (200 mg mL^{-1})	656.0±8.8	638.8±8.9** (2.62)	580.9±9.2** (11.44)
Insulin (1 U mL^{-1})	689.5±8.6	625.1±6.9** (9.34)	561.5±7.6** (18.56)
MEHB (200 mg mL^{-1})+insulin (1 U mL^{-1})	690.8±6.7	607.3±8.4** (12.08)	500.4±5.5** (27.56)

MEHB: Methanolic extract of *Hiptage bengalensis*, All the values of Mean±SD, n = 6, *,**p<0.05, 0.01 vs. control, In brackets % Uptake of Glucose levels were mentioned

100 and 200 mg mL^{-1} after 3 h of its incubation. This indicates the mechanism of action of MEHB for its antidiabetic activity. The combination of MEHB (200 mg mL^{-1}) and insulin produced an additive effect on glucose uptake pattern by the diaphragm (Table 6).

DISCUSSION

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency which produces inadequate glucose control and leads to acute and chronic complications. Premature and extensive arteriosclerosis, involving renal, peripheral and cardiovascular vessels, remains the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart disease. A reduction in serum lipids, the LDL and VLDL fraction and TG, should be considered as being beneficial for the long-term prognosis of these patients (Chattopadhyay and Bandyopadhyay, 2005). Glucose control is essential but this provides only minimal benefit with respect to CHD prevention. An ideal treatment for diabetes would be a drug that not only controls the glycemic levels but also prevents the development of arteriosclerosis and other complications of diabetes (Halliwell and Gutteridge, 1985). There has been an increasing demand for alternative medicine especially, the consumption of botanicals have been increasing rapidly worldwide, mostly because of the supposedly low cost, easy availability and less frequent side-effects when compared to modern Western medicine (Sharma *et al*., 2010).

Diabetes mellitus is considered as one of the five leading causes of death in the world (Vats *et al*., 2004). Considerably, large number of hypoglycemic/antidiabetic plants and herbs are known through folklore but their introduction into modern therapy waits pharmacological testing by modern methods. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative (Lenzen, 2008). Alloxan is toxic glucose analogue, when administered to rats and many other species which selectively destroys insulin-producing beta cells in the pancreas resulting in insulin-dependent diabetes mellitus (Alloxan Diabetes) with characteristics similar to type 1 diabetes in humans (Lenzen, 2008).

The present study results suggest that the methanolic extract of *Hiptage bengalensis* leaves exhibited significant antihyperglycemic activity in alloxan induced diabetic rats. Fasting blood glucose level in diabetic rats is an important basal parameter for monitoring diabetes (Maiti *et al*., 2005) and it has shown that the MEHB causes the antihyperglycemic effect by reducing ($p<0.05$) the fasting blood glucose level in a dose dependant manner.

The significant decrease in the levels of fasting blood glucose in diabetic rats treated with the MEHB may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose. These results support the study conducted by the (Erah *et al*., 1996). The antihyperglycemic activity of the MEHB was due to the regeneration of pancreatic cells that were partially destroyed by alloxan and potentiation of insulin secretion from surviving beta cells of the islets of langerhans (Suba *et al*., 2004). The methanolic extract may increase secretion of insulin from beta cells of pancreas and results in of this increased secretion of insulin stimulate fatty acid biosynthesis and also the incorporation of fatty acids into triglycerides in the liver and adipose tissue (Best and Taylor, 1989).

Hyperlipidemia is a recognized consequence of diabetes mellitus (Pushparaj *et al*., 2000; Sharma *et al*., 2003). Previous studies suggested that hyperglycemia and hyperlipidemia are the common characteristics of Alloxan-induced diabetes mellitus in experimental rats (Saravanan and Pari, 2003; Luo *et al*., 2004; Yadav *et al*., 2005). High level of TC and LDL are major coronary risk factors (Temme *et al*., 2002). Further, the studies suggested that TG itself is independently related to coronary heart disease (Bainton *et al*., 1992). The abnormalities in lipid metabolism lead to elevation in the levels of serum lipid and lipoprotein that in turn play an important role in occurrence of premature and severe atherosclerosis which affects patients with diabetes (Ravi *et al*., 2005).

Moreover, supplementation of methanolic extract of *Hiptage bengalensis* produced a significant beneficial effects in the lipid profile in diabetic rats by reducing triglycerides, total cholesterol and increasing HDL-C levels significantly ($p<0.05$) (Table 2-4). This effect may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin (Sharma *et al*., 2003). It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism. The increase in HDL may slow down the atherosclerotic process (Nofer *et al*., 2002). Increased levels of HDL (cardioprotective lipid) after administration of methanolic extract of *H. bengalensis* extract concluded its cardio protective effect.

Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of the glucose (Krishnakumar *et al*., 2000). The methanolic extract may cause the regeneration of the beta cells of the pancreas and potentiation of insulin secretion from surviving beta cells; the increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this context, a number of other plants have been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects (Ramalingam and Pari, 2005; Fernandes *et al*., 2007). Further study on the plants could be extended for the isolation and structure determination of the antidiabetic principle or principles.

The present study results also indicated the dose dependant antidiabetic activity of MEHB in chick model. Some studies to investigate whether diabetes model can be made by treatment of Streptozotocin (STZ) in chick embryos and this model can be used to predict the effect of drug (Yashiyama *et al*., 2005). In their study STZ (0.3 mg egg^{-1}) was injected into the albumen of fertile egg on 14th day of incubation, level of blood glucose significantly and they concluded that STZ treated embryos was applicable to evaluate human insulin and anti diabetes drugs an experimental diabetes model. But they concluded that the diabetes can be induced in chick embryos with STZ (Yashiyama *et al*., 2005). There are some other who tried to calculate lethal dose for Alloxan and STZ injected systematically in chicks but failed to calculate dose of Alloxan and STZ to cause diabetes in chicks (Danby *et al*., 1982). So, it was decided to continue the study based on their observations by developing diabetic chicks as a model by inducing alloxan, STZ in chick embryos.

Administration of alloxan (0.6 mg/30 g egg) into the egg sac at 14th days of incubation resulted in the development of chicks with diabetic condition and the blood glucose levels were found greater than 300 mg dL^{-1}. This indicated the successful induction of diabetes in chicks. Administration of MEHB at doses of 100 and 200 mg kg^{-1} to these diabetic chicks reduced the fasting blood glucose levels ($p<0.01$) to normal range (230.8±13.9) (Table 5).

In the present *in vitro* study of glucose uptake method results indicate that methanolic extract of HB significantly increase the glucose uptake and results were similar to that of insulin. These findings suggest that the methanolic extract of HB might have direct metformin like insulin activity which enhances the peripheral utilization of glucose and have extra pancreatic effect. The uptake of glucose by the skeletal muscles has been potentiated in presence of insulin and MEHB.

CONCLUSION

In conclusion, the present study results indicated that Methanolic extract of leaves of *Hiptage bengalensis* was endowed with antidiabetic activity. Since this plant possess promising protective effect against diabetes, it needs comprehensive investigations for developing a safe and effective herbal drug. Further research is required to isolate the biomolecules responsible for the antidiabetic activity.

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