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PRECLINICAL EVALUATION OF ANTIDIABETIC AND HYPOLIPIDEMIC EFFECTS OF HIBISCUS TILIACEUS

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ABSTRACT

Objective: The study was carried out to evaluate preclinical antidiabetic and hypolipidemic effects of ethyl acetate fraction of *Hibiscus tiliaceus* methanolic leaves extract (HTEF) in alloxan induced diabetic rat by administering oral doses (200 and 400 mg/kg body weight). **Methods:** HTEF was orally for 21 days and its effect on blood glucose, lipid profile, liver profile and renal profile were examined in in normal and alloxan induced diabetic rats. Histopathological study of normal and diabetic rat organs (pancreas, liver and kidney) were also carried out after extract treatment. **Results:** Oral administration HTEF (200 and 400 mg/kg body weight) and glibenclamide (10 mg/kg) daily for 21 days reduced blood glucose significantly. The extract also Improved kidney, liver functions and hyperlipidaemia due to diabetes and showed favorable effect on the histopathological changes of the pancreas, liver and kidney in alloxan induced diabetes. **Conclusion:** The methanolic extract of *H. tiliaceus* reduces blood glucose level and improves lipid metabolism. The extract also shows improvement in parameters like liver profile and renal profile as well as regeneration of β-cells of pancreas in diabetic rats. Histopathological studies also show favorable effect to inhibit the histopathological changes of the pancreas and kidney in alloxan induced diabetes.

KEYWORDS: Alloxan, Antidiabetic, *Hibiscus tiliaceus*, Histopathology, Hypolipidemic, Pancreas, Kidney, Liver, Rat.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The prevalence of diabetes is projected to increase from 171 million in 2000 to 366 million in 2030. [1] Synthetic antidiabetic agents can

produce serious side effects and they are not suitable for use during pregnancy. In view of the adverse effects associated with the synthetic drugs and as plants are safer, cheaper and much effective, conventional antidiabetic plants can be explored. [2] Many traditional plant treatments for diabetes mellitus are used throughout the world. [3-4] Furthermore, World Health Organization has also recommended the evaluation of traditional plant treatments for diabetes.

Hibiscus tiliaceus L. is a plant of family Malvaceae. It is a mangrove plant growing in tropical Asia and abundant in forests. In folk medicine, the leaves of this plant used to treat fevers, soothe coughs, ulcer, wounds and various skin diseases. [5] In traditional Indian system of medicine *H. tiliaceus* had been used as febrifuge, laxative, resolvent and emollient. An infusion of leave is employed to wash ulcer and wounds. [6-8] The plant also showed antinociceptive and anti-inflammatory effects. [9] Traditionally, the plant has been used for diabetes. [10] The methanolic flower extract of the plant shows antidiabetic and hypolipidemic potentials in streptozotocin induced diabetic wistar rat. [11] However, no antidiabetic activity on leaves extract is reported hence, an attempt to the antidiabetic, hypolipidemic and histopathological analysis of ethyl acetate fraction of *Hibiscus tiliaceus* methanolic leaves extract in alloxan induced diabetic rat.

MATERIAL AND METHODS

Plant material

Hibiscus tiliaceus leaves were collected during month of August from the campus of Kurukshetra University, Kurukshetra, India and were identified by Dr. B.D. Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, India. A voucher specimen of the plant is preserved in the Institute of Pharmaceutical Sciences, Kurukshetra University (No. IPS/KUK/HT/2009).

Extract preparation and fractionation

The leaves were dried under shade and powdered to coarse particles. The powdered material was extracted with methanol in Soxhlet extraction apparatus at 60°C. The extract was dried at 45 ^{0}C in rotary evaporator to produce a semisolid mass. The solid extract was suspended in water and successively extracted with hexane, ethyl acetate and n-butanol by separating funnel and stored in airtight containers in refrigerator below 10 ^{0}C.

Chemicals

Alloxan was purchased from Loba chemie Pvt. Ltd. Mumbai, India. Total cholesterol (TC), serum high-density lipoprotein (HDL), serum Creatinine (SC), serum urea (SU), serum alkaline phosphate (ALP), alanine transaminase (ALT), serum aspartate transaminase (AST) and triglyceride (TG) standard kits were obtained from Erba diagnostics Mannheim Gambh, Germany. Blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany. All reagents used in study were analytical grade.

Animals

Wistar rat of either sex, weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions i.e. ambient temperature of 22 ± 2 ^{0}C and at 45–55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. All the studies were conducted in accordance with the Animal Ethical Committee of the University.

Induction of diabetes

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Loba Chemie, Bombay; 150 mg/kg i.p.) in sterile saline. Twelve days after Alloxan injection, rats with blood glucose level of >200 mg/dl were separated and used for the study. Blood glucose levels were measured using blood glucose test strips with elegance glucometer (Frankenberg, Germany) at weekly intervals till the end of study (i.e. 3 weeks). Blood glucose estimation and body weight measurement were done on 0, 7, 14 and 21 day after administration of extract orally.

Experimental design

Overnight fasted rats were divided into five groups and for each group six animals and treated orally once a day for 21 days as follows:

Group I. Normal healthy control: given only vehicle (Tween 80, 1% v/v)

Group II. Diabetic control: given only vehicle (Tween 80, 5% v/v)

Group III. Diabetic rats given HTEF (200 mg/kg b.w.)

Group IV. Diabetic rats given HTEF (400 mg/kg b.w.)

Group V. Diabetic rats given Glibenclamide (10 mg/kg b.w.).

Biochemical parameters

Blood glucose was measured with elegance glucometer (Frankenberg, Germany) at weekly intervals i.e. 0, 7, 14 and 21 day after daily administration of extract orally. After blood glucose estimation on day 21, whole blood was collected by cardiac puncture under mild ether anesthesia from rats. Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase, HDL and total proteins levels were also evaluated in normal and alloxan induced diabetic rats.[12] Serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were measured by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits. [13-14]

Statistical analysis

All values of results are presented as mean ± standard error of mean (S.E.M.) The statistical analysis involving two groups was evaluated by means of Student's t-test whereas one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the p <0.05 values.

RESULTS

Antidiabetic activity: Single dose intra-peritoneal (i.p) treatment of rats with alloxan monohydrate (150 mg/kg) significantly (p <0.001) increases the blood glucose as shown in table 1. The antidiabetic effect of ethyl acetate fraction of methanolic extract of *H. tiliaceus* (HTEF) was studied in alloxan induced diabetic rats. After the daily oral administration with HTEF (200 and 400 mg/kg, p.o.), for 21 days, significant decreased (p<0.001) in the blood glucose a level was observed in the diabetic rats (Table 1).

Effect on lipid profile

Diabetes is also associated with altered lipid profile. There was a significant increase of serum total cholesterol, triglycerides, and significant decrease in HDL cholesterol in diabetic rats as compared to that of normal control. The standard drugs as well as HTEF (200 and 400 mg/kg) significantly decreased (p<0.05) the levels of cholesterol and triglycerides HDL cholesterol level was enhanced (Table 2) after 21 days HTFE treatment.

Effect on other biochemical parameters

AST, ALT and ALP level were increased in diabetic rats which is responsible for the liver damage. The rats treated with HTEF showed significant (p < 0.001) reduction in the elevated

levels of liver enzymes (transaminase) in a dose dependent manner. Bilirubin level was also decreased in diabetic rats after the treatment. Total protein level was decreased significantly in diabetic rats and after 21 days treatment, the protein level was increased significantly ($p < 0.01$) as shown in table 3. Kidney function markers like creatinine and urea were elevated in the alloxan induced diabetic rats when compared with the normal rats. HTEF reduced the levels in dose dependent manner (Table 4).

Histology of organs

Photomicrographs of liver (Figure 1) showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein (A). In case of group II diabetic rats, the normal lobular structure was preserved. The central vein was prominent and prominently congested. Focal areas of hemorrhage were also seen. Fatty change was evident (B). The hepatocytes portal tracts and central veins were appeared normal after treatment with HTEF 400 mg/kg (C).

Histology of pancreas (Figure 2) showed normal acini, and normal cellular population in the islets of Langerhans in pancreas of vehicle-treated rats (D). In diabetic animals treated extensive damage to islets of langerhans and reduced dimensions of islets were observed in diabetic rats (E). HTEF 400 mg/kg had partially restorated the normal cellular population and enlarged size of β-cells (F). Photomicrographs of kidney (Figure 3) in normal animals showed normal structure (G). In diabetic rats, kidney section showed mild thickening of the basement membrane of the arterioles of glomeruli along with mild change of density of mesangium (H). After HTEF 400 mg/kg treatment, these changes were improved towards normal condition (I).

DISCUSSION

Alloxan has a destructive effect on the beta cells of the pancreas and causes massive reduction in insulin release, thereby inducing hyperglycaemia. [15] Insulin deficiency leads to various metabolic alterations in the animals such as increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases etc. [16-17] HTEF and glibenclamide both were found to reduce the hyperglycemia significantly in alloxan- induced diabetic animals during 21 days treatment. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. [18] Repeated administration of the HTEF for 21 days significantly ($p<0.05$) decreased cholesterol and triglyceride levels. This hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis.[19]

Liver enzymes e.g. AST, ALT and ALP level were increased in diabetic rats which is responsible for the liver damage or hepatic diseases. [20] The elevated level of these enzymes was significantly reduced by HTEF and glibenclamide treatment. Serum protein and bilirubin levels were also reduced by the treatment. Serum urea and creatinine are the marker of renal diseases. HTEF improved renal functions in diabetic rats by reducing serum urea and creatinine levels. Histopatholigical studies of tissues of organs (liver, pancreas and kidney were undertaken and it was found that HTEF was non-toxic and regenerated the toxic effect of alloxan.

TABLE 1. Effect of HTEF on the blood glucose and insulin levels in alloxan diabetic rat (A-D).

Groups/Treatments	Blood glucose level (mg/dl)			
	Initial day	Day 7	Day 14	Day 21
I: Normal + Vehicle	112.23 ± 2.5	112.34 ± 2.3	113.72 ± 3.22	113.92 ± 3.4
II: A-D + vehicle	253.52 ± 2.45	296.54 ± 4.35a	325.46 ± 4.27	389.24 ± 4.34a
III: A-D + HTEF (200 mg/kg)	257.35 ± 2.43	232.26 ± 2.25*	187.53 ± 2.34*	126.23 ± 3.28*
IV: A-D + HTEF (400 mg/kg)	288.53 ± 2.5	205.23 ± 2.5*	142.22 ± 3.24*	119.58 ± 3.27**
V: A-D + Glibenclamide (10 mg/kg. b.w.)	255.24 ± 2.28	201.23 ± 3.52	130.31 ± 2.34*	116.42 ± 2.8**

N=6, Data represent means ± S.E.M. *p<0.05, **p <0.001, When groups III, IV and V compared with diabetic control i.e. group II, N= Numbers of animals in each group.

TABLE 2 - Effect of HTEF on lipid profile (mg/dl) in alloxan induced diabetic rats.

Groups/Treatments	Total Cholesterol	Triglycerides	HDL cholesterol
I: Normal	87.28 ± 3.8	82.42 ± 5.16	37.32 ± 2.9
II: A-D	254.73 ± 7.6 a	150.52 ± 4.71	28.23 ± 2.2 a
III: A-D + HTEF (200 mg/kg)	122.45 ± 2.38*	112.24 ± 2.32*	34.25 ± 2.24*
IV: A-D + HTEF (400 mg/kg)	88.33 ± 2.64*	83.57 ± 3.25**	44.25 ± 3.35*
V: A-D + Std. (10 mg/kg.b.w.)	98.72 ± 5.3*	83.47 ± 4.5*	45.28 ± 4.8**

Data represent means ± S.E.M. *p<0.05, **p <0.001

Table 3: Effect of HTFE on liver parameters in normal and diabetic rats.

Groups	Total Protein (g/dL)	Bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
I	7.26 ± 2.18	0.45 ±1.24	43.22 ± 2.34	59.35 ± 3.49	123.35 ± 3.43
II	5.26 ± 1.29	0.94 ±1.29 [a]	102.26 ± 4.87	113.23 ± 3.45	198.26 ± 4.37 [a]
III	5.47 ± 3.47[*]	0.53 ± 1.27[*]	64.35 ± 3.42	60.32 ± 2.84[*]	142.53 ± 3.38[*]
IV	8.33 ± 2.33[**]	0.44 ±1.39[**]	43.24 ± 3.16[*]	58.53 ± 4.32[**]	126.44 ± 1.85[**]
V	7.21 ± 1.25[*]	0.38 ± 1.83[*]	45.56 ± 3.54[**]	58.86 ± 3.58[*]	125.25 ± 3.25[**]

Data represent means ± S.E.M., [*]p<0.05, [*]p<0.001, When groups III, IV and V compared with diabetic control i.e. group II, [a]p<0.05, When Group II compared with group I.

Table 4: Effect of HTEF on kidney parameters in normal and diabetic rats.

Groups/Treatments	Serum Urea (mg / dl)	Serum Creatinine (mg/dl)
I: Normal	30.25 ± 1.58	0.63 ± 1.34
II: A-D	59.24 ± 1.57	0.97 ± 0.54 [a]
III: A-D + HTEF (200 mg/kg)	37.57 ± 3.52	0.75 ± 2.34[*]
IV: A-D + HTEF (400 mg/kg)	36.7 ± 1.34[*]	0.67 ± 3.74[**]
V: A-D + Std. (10 mg/kg.b.w.)	35.35 ± 0.87[*]	0.65 ± 0.62[**]

Data represent means ± S.E.M., [*]p<0.05, [*]p<0.001, When groups III, IV and V compared with diabetic control i.e. group II, [a]p<0.05, When Group II compared with group I

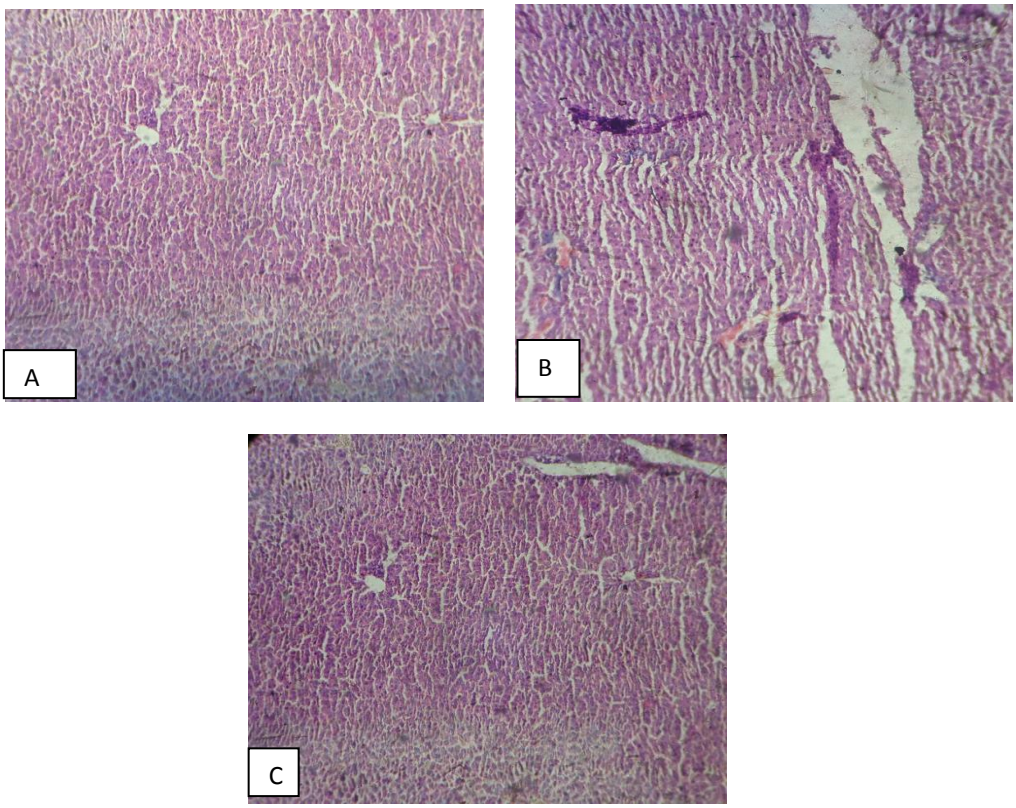

Figure 1: Effect of HTEF on rat liver (A: Normal, B: Diabetic, C: A-D + HTEF 400 mg/kg).

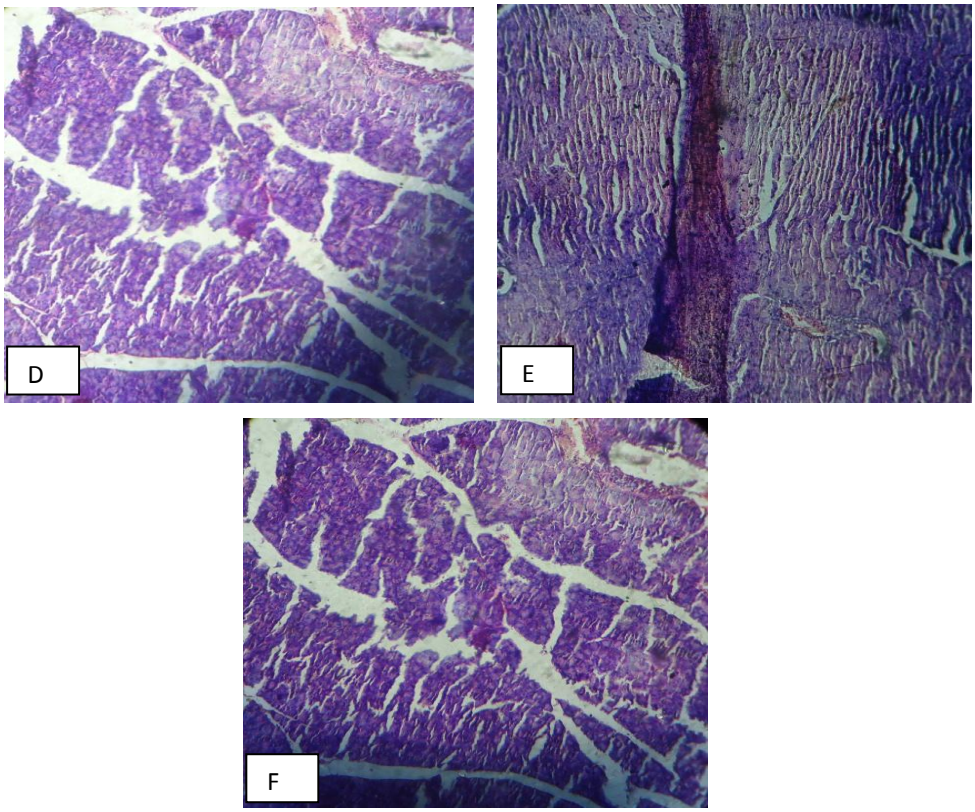

Figure 2: Effect of HTEF on rat pancreas (D: Normal, E: Diabetic, F: A-D + HTEF 400 mg/kg).

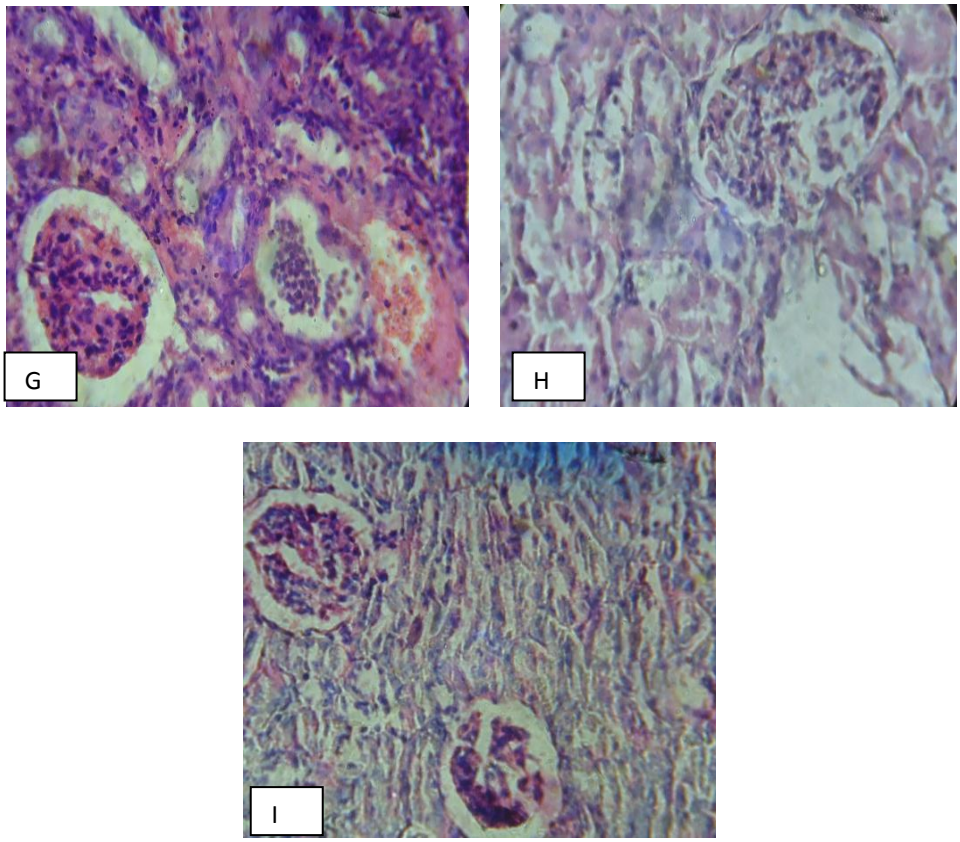

Figure 3: Effect of HTEF on rat kidney (G: Normal, H: Diabetic, I: A-D + HTEF 400 mg/kg).

CONCLUSION

The result of the present study showed that HTEF reduced hyperglycemia and hyperlipidemia diabetes-induced rats. The fraction also improved kidney and liver functions. HTEF has favorable effect to inhibit the histopathological changes of the pancreas and kidney in alloxan induced diabetes. It has also inhibited the histopathological changes of the pancreas, liver and kidney in alloxan induced diabetes. Although the exact chemical compounds responsible for the antihyperglycemic and antihyperlipidemic effects of *H. tiliaceus* need to explore in further studies.

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