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Evaluation of Insulin Potentiating Activity of Ethanolic Root Extract of *Boerhavia Diffusa* in Streptozotocin –Induced Diabetic Rats

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ABSTRACT

To evaluate anti-diabetic activity, insulin potentiating activity of ethanolic root extract of *Boerhavia diffusa* (BD) in streptozotocin induced diabetic rats and isolation of active fraction from the extract. The ethanolic root extract was tested for its Acute toxicity studies and insulin potentiating activity and the dosages were selected as 200mg & 300mg /kg body weight for the activity screening. Glibenclamide 0.5 mg/Kg body wt. was used as standard and Insulin 10 units/Kg body wt. was used concurrently with the extracts for 14 days. At 0, 5, 10, 15 day fasting blood glucose level measured and 15th day serum lipid levels and pancreas histopathology were observed. Ethanolic extract (300mg/kg) p.o with insulin treated group exhibited significant anti hyperglycemic activity in streptozotocin induced diabetic rats. It also improved the condition of diabetes parameters like body weight gain, serum cholesterol, triglycerides, HDL, LDL and VLDL when compared with the diabetic control group. Butanol fraction showed significant decrease in blood glucose levels compared with the other fractions of *BD*. Histopathological studies indicated that there is dose dependent effect of the *BD* extract on the preservation of architecture of pancreatic cells. The results indicated that the *Boerhavia diffusa* possesses antidiabetic and insulin potentiating activity.

Keywords: Antidiabetic activity, *Boerhavia diffusa*, streptozotocin, Insulin

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by absolute or relative deficiency in insulin secretion or action and is associated with chronic hyperglycemia, which can cause serious health complications including ketoacidosis, end stage renal disease, heart disease, stroke, blindness and a variety of debilitating neuropathies [1]. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2050 [2]. The pathogenesis of diabetes is managed by insulin and oral hypoglycemic drugs.

Type 1 diabetes is caused by a deficiency of insulin secretion from β-pancreatic cells. On the other hand, Type 2 diabetes is closely associated with obesity and is characterized by an initial phase of progressive insulin resistance, with an ensuring reduction in the ability of the pancreatic hormone to promote peripheral glucose disposal and to suppress hepatic glucose output [3].

In Asia and Africa, there are limitations on presently available therapeutic options for diabetes, such as oral hypoglycemic agents and insulin. In such circumstances, herbal medicines for the treatment of diabetes become significant. Available literature reveals that more than 400 plant species have been claimed to have anti-hyperglycemic activity.

Boerhaavia diffusa L. belongs to the family Nyctagenaceae. Its leaves are used as vegetables and the root juice is used to cure asthma, urinary disorders, rheumatism, liver disorders and diabetes. It has a long history of uses by indigenous and tribal people and in Ayurvedic and Natural Herbal Medicine [4]. It has been found to possess diuretic action, anti-inflammatory, antifibrinolytic, anticonvulsant and hepatoprotective, antidiabetic, anticancer, antioxidant, antiviral activities [5]. The present study was undertaken to verify the claim and evaluate the antidiabetic activity of the plant *Boerhavia diffusa.*

MATERIALS AND METHODS

Chemicals

Streptozotocin obtained from sisco research laboratory Mumbai, glibenclamide was obtained from the sigma chemical. All other chemicals procured from SD fine chem. Ltd.

Plant Material

The roots of *Boerhavia diffusa* were directly purchased from the reputed ayurvedic shop and it is authentified by Dr. V. C. Guptha, M.Sc., Ph.D Ex Deputy Director (Botany) in Central Research Institute for Unani Medicines Dept. of Ayush. The roots were cleaned, shade dried at room temperature, coarse powdered and then extracted with 95% ethanol by using Soxhlets

apparatus for 8hrs at 70^{0}C. Thereafter, the extract was concentrated evaporation. The dried crude extract was stored in refrigerator below 10^{0}C for further studies.

Preliminary Phytochemical Screening

The photochemical screening of BD root contains phenols, carbohydrates, glycosides, alkaloids, sterols and flavonoids.

Animals

Male Wistar rats weighing 150-280 g and male Swiss albino mice (19-31g) were used with the approval of the Institute Animal Ethics Committee. Animals were fed a standard pellet (Lipton India, Ltd) and water *ad-libitum* and maintained at 24- 28^0 C temperature, 60-70% relative humidity, and 12 h day and night cycle. Animals described as fasted were deprived of food for 16 h but had free access to water. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Acute Toxicity Study

The alcohol extract was tested for its acute toxicity in mice. To determine acute toxicity of a single oral administration of the herbal drug, different doses of the drug (0.5, 1.0, 1.5 and 2 g/kg) were administrated to four groups of mice (2 mice were used for each group, control mice received distil water). Mortality and general behavior of the animals were observed periodically for 14days. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [6].

Induction of Diabetes to Experimental Rats

Diabetes was induced in overnight fasted rats by intraperitonial injection of streptozotocin (30mg/kg, *i.p.*) [7] dissolved in 0.01 M cold sodium citrate buffer (pH 4.5) immediately before use. The rats were then given 5% w/v glucose solution in feeding bottles for the next 24 h in their cages to prevent hypoglycemia. After 48 hr, rats with marked hyperglycemic fasting blood glucose > 200 mg/dl were selected and used for the study [8].

Collection of Blood Samples

Blood samples were collected by end tail vein cutting method and retro orbital puncture.

Experimental Design and Biochemical Analysis

In the present experiment a total of 48 rats were used. The rats were divided in to eight groups each contains six rats. One was control and remaining seven groups were streptozotocin

induced diabetic rats. Group I-Normal control animals received 0.5ml distilled water per oral, Group II-Diabetic control received 0.5ml distilled water per oral, Group III and IV-Received ethanolic root extract at a dose of 200 and 300 mg/kg per oral, Group V-Received standard drug, glibenclamide at a dose of 0.5mg/kg p.o, Group VI and VII-Received extract at a dose of 200 and 300mg/kg p.o and insulin at a dose of 10U/kg s.c, Group VIII-Received insulin at a dose of 10U/kg s.c. for 14 days. At the 0, 5, 10, 15 day estimate the fasting blood glucose levels by using one touch electronic glucometer using glucose strips. Body weights were measured at initial and final days.

On day 15 blood was collected by retroorbital puncture and separate the serum and analysed for serum cholesterol by Trinder CHOD/POD End point method [9], serum triglycerides by GPO/PAP METHOD [10], serum HDL [11], serum LDL, serum VLDL were estimated.

The pancreas from the each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and processed by the paraffin technique. Sections pancreas of 5µ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination. The photographs of histological studies were presented in Fig. 3

Isolation of an Active Fraction

The ethanol extract was suspended in water and successively extracted with petroleum ether, chloroform, ethyl acetate and butanol. Each fraction was dried free of solvent and the yield was determined. The dose selected for glucose tolerance test in each case was based on the yield from the ethanol extract. Since 400 mg/kg body weight is double the optimum dose the yield from 400 mg alcohol extract in the case of each fraction was administrated per kg, body weight.

Glucose Tolerance Test

Briefly, overnight fasted rats were divided in to 5 groups. One group was kept as control which received 5% Tween 80 (0.5 ml, *p.o.*), group 2 received petroleum ether fraction (160 mg/kg) in 0.5% Tween 80 (0.5 ml, *p.o.*), groups III, IV and V received chloroform fraction (25 mg/kg), ethyl acetate fraction (75 mg/kg) and butanol fraction (100mg kg) respectively in 5% Tween 80 (0.5 ml. *p.o.*). The rats of all the groups were loaded with glucose (3 g/kg, *p.o.*) 30 min after the administration of the drugs (fractions). Blood sugar levels were measured at 30, 90 and 150 min after glucose loading by using digital glucometer.

Statistical Analysis

The data were expressed as Mean ± SEM. Statistical evaluation was done by One way ANOVA followed by dunnett's post analysis using Graph Pad Prism version 5.0, USA. The minimum level of significance was fixed at $p < 0.05$. Statistical significance was divided as recommended by Graph Pad Prism software.

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RESULTS AND DISCUSSION

Acute Toxicity Studies

This study showed no mortality up to the dose of 2000 mg/kg body weight. So, the extracts safe for long term administration.

Effect of *Boerhavia diffusa* root extract on body weight in streptozotocin induced diabetic rats.

Table 1: Effect of herbal extract on Streptozotocin induced diabetic rats

Gr. No	Treatment	Body weight(gm)	
		Initial	Final
1	Normal control	241.66±6.103	255.83±4.175
2	Diabetic control	196±16.653	143.83±11.146
3	Etanolic extract 200mg/kg	217±10.49	174±10.44
4	Ethanolic extract 300mg/kg	225±13.346	185.83±14.55
5	Glibenclamide 500mg/kg	202±15.282	214±15.154**
6	Ethanolic extract 200mg/kg+Insulin10U/kg	204.83±18.376	195.16±22.48
7	Ethanolic extract300mg/kg+Insulin10U/kg	243.33±14.481	244.66±12.465***
8	Insulin10U/kg	251.16±6.877	263.16±5.759***

Values are mean ± SEM, N=6: [a]P <0.001, [b]P <0.01, [c]P <0.05 vs. diabetic control.

(Table 1) show in normal control group the mean body weight (±SEM) on day 0 was 241.66±6.10 and on day 15 weight gain was 255.83±4.17. In diabetic control mean body wt on day 0 was 196±16.65 and on day 15 there was significantly (P<0.001) decrease in body wt 143.83±11.14. In the Group III and IV mean body weights on day 0 were 217±10.49, 225±13.34 and on day 15 decrease in body weights but not significantly (174±10.44, 185.83±14.55, P>0.05). In glibenclamide group mean body wt on day 0 was 202±15.28 and on day 15 there was significantly (P<0.01) increase in body wt 214±15.15. In the Group VI mean body wt on day 0 was 204.83±18.37 and on day 15 not significantly (P>0.05) decrease in body wt 195.16±22.48. In Group VII and VIII mean body weights on day 0 were 243.33±14.48, 251.16±6.87 and on day 15 mean body weights were increased significantly (P<0.001) 244.66±12.46, 263.16±5.75.

The antihyperglycemic effect of the extract on the fasting blood sugar level of diabetic rats was shown in the (fig. 1). In normal control group found to be stable in their fasting blood glucose levels. In diabetic control there was no change in FBG levels from day 0 to 15. In Group III and IV percentage reduction of FBG from 0 to 15 day was 67.75 and 67.28 with high significant (P<0.001). In Group V percentage reduction of FBG from 0 to 15 day was 56.9 with high significance (P<0.001). In Group VI and VII percentage reduction in the FBG from 0 to 15 was 71.86 and 71.45 with high significance (P<0.001). In the Group VIII percentage reduction in FBG from 0 to 15 was 61.99 (Table 2).

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Table 2: Effect of *Boerhavia diffusa* root extract on fasting blood glucose levels in streptozotocin induced rats.

Gr. No	Treatment	Fasting blood glucose level mg/dl (% inhibition)			
		Day0	Day5	Day10	Day15
1	Normal control	84±2.01	92.66±5.02	88.5±5.19	94±4.97
2	Diabetic control	360.5±30.94	348±65.06	420.33±16.81	384.33±15.36
3	Etanolic extract 200mg/kg	377.33±32.04	99.5±17.13[a] (73.63)	209.16±48.39[a] (44.56)	121.66±5.84[a] (67.75)
4	Ethanolic extract 300mg/kg	342.33±29.09	138.33±41.86[b] (59.59)	158.33±49.52[a] (53.74)	112±23.05[a] (67.28)
5	Glibenclamide 500mg/kg	353.83±34.41	333±32.38 (5.88)	228.5±13.61[a] (35.42)	152.5±9.73[a] (56.9)
6	Ethanolic extract 200mg/kg+Insulin10U/kg	396.66±61.12	278±31.11 (29.91)	247.5±43.01[b] (37.6)	111.5±22.93[a] (71.86)
7	Ethanolic extract300mg/kg+Insulin10U/kg	314.66±13.94	131.33±18.59[a] (58.26)	111.66±3.27[a] (64.51)	89.83±14.01[a] (71.45)
8	Insulin10U/kg	242.5±3.01	104.5±15.07[a] (56.9)	100.66±3.98[a] (58.49)	92.16±2.96[a] (61.99)

Values are mean ± SEM, N=6: [a]P <0.001, [b]P <0.01, [c]P <0.05 vs. diabetic control.

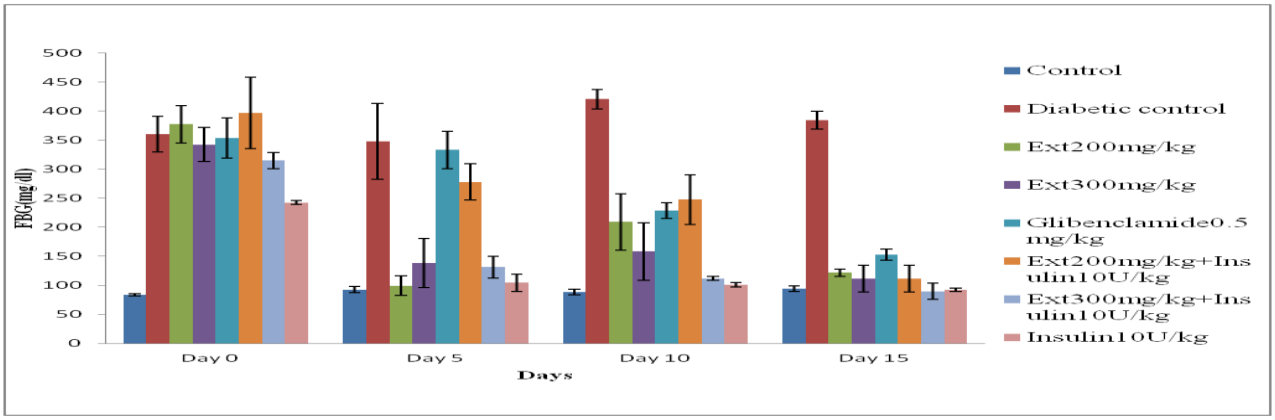

Figure 1: Effect of *Boerhavia diffusa* root extract on fasting blood glucose levels in diabetes rats

Table 3: Effect of *Boerhavia diffusa* root extract on lipid levels in streptozotocin induced rats.

Gr. No	Treatment	Lipid levels mg/dl				
		TC	TG	HDL	LDL	VLDL
1	Normal control	79.16±2.4	72±5.48	54±4.88	10.23±5.90	14.35±1.1
2	Diabetic control	143±2.543	149.16±6.92	28.83±1.01	84.33±3.10	29.83±1.38
3	Ethanolic extract 200mg/kg	66.16 ±1.35[a]	34.16 ±1.74[a]	40.5 ±0.76[c]	18.8 ±1.23[a]	6.83 ±0.34[a]
4	Ethanolic extract 300mg/kg	74.16±1.6[a]	37.33±1.85[a]	46.16±1.74[a]	20.53±2.48[a]	7.46±0.37[a]
5	Glibenclamide 500mg/kg	97±1.03[a]	86.66±1.25[a]	55.33±3.77[a]	24.33±3.45[a]	17.33±0.25[a]
6	Ethanolic extract 200mg/kg+Insulin10U/kg	78±3.01[a]	59.5±2.46[a]	52.16±2.45[a]	13.93±2.45[a]	11.9±0.49[a]
7	Ethanolic extract 300mg/kg+Insulin10U/kg	68±5.10[a]	51.33±7.63[a]	45.5±4.44[a]	9.86±0.98[a]	10.46±1.47[a]
8	Insulin10U/kg	55.16±3.35[a]	51.16±4.25[a]	40.16±2.90[c]	4.76±3.11[a]	10.1±0.90[a]

Values are mean ± SEM, N=6: [a]P <0.001, [b]P <0.01, [c]P <0.05 vs. diabetic control.

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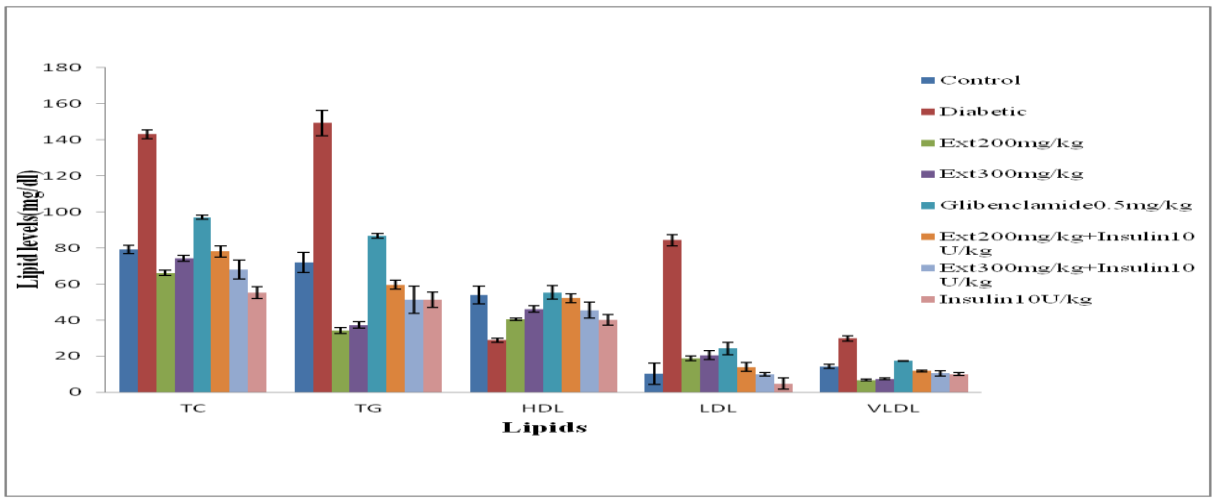

Figure 2: Effect of *Boerhavia diffusa* root extract on lipid levels in diabetes rats

Serum cholesterol, serum triglycerides, serum HDL, serum LDL, serum VLDL levels were decreased significantly in extract and insulin treated groups compared with the diabetic control group was shown in (Table 3).

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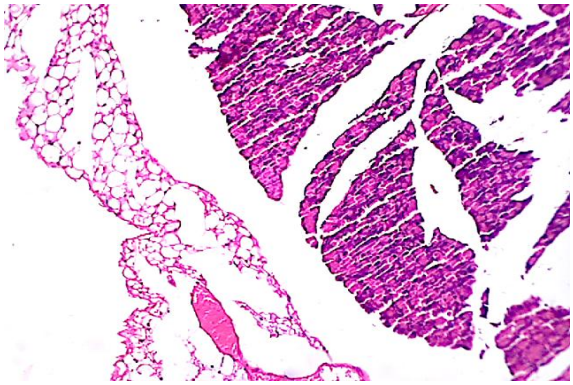

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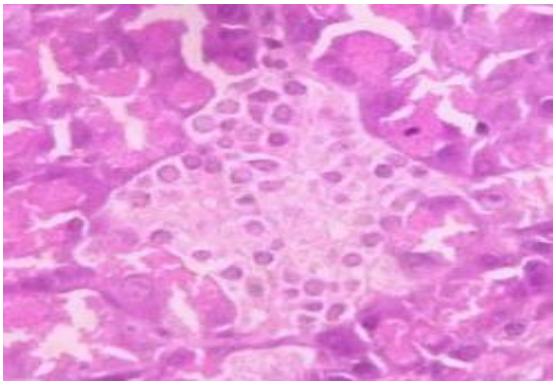

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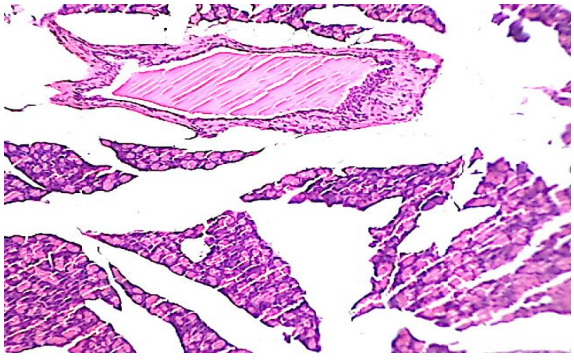

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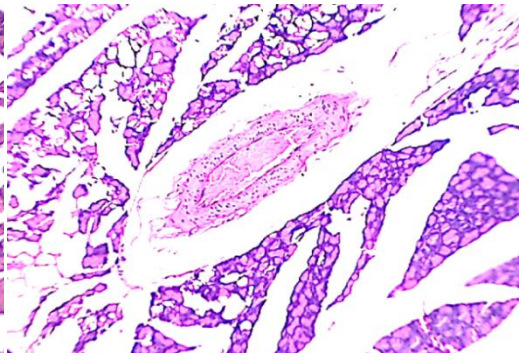

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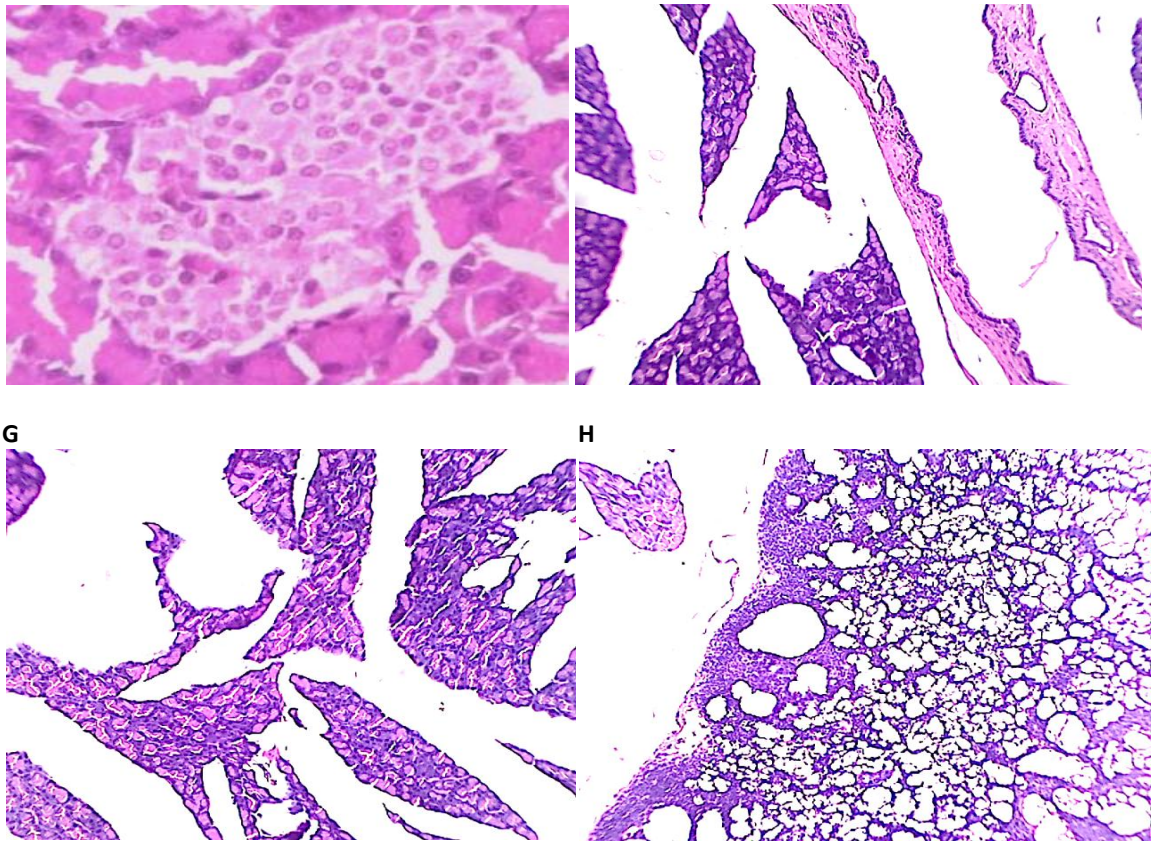

Figure 3: Photomicrographs of rat pancreas stained by haematoxylin and eosin

Photomicrographs (figure 3) showed normal architecture, no damage to pancreatic cells in normal control group. Extensive damage to pancreatic cells with destroyed architecture, restoration of pancreatic cells by glibenclamide and insulin was also shown. Partial restoration of pancreatic cells by extract treated groups and along with insulin treated groups.

Isolation of an Active Fraction

Table 4: Oral glucose tolerance test of different fractions of root ethanol extract of *Boerhavia diffusa* in normal rats

Gr. No	Treatment	Blood glucose levels (mg/dl) at different time intervals (min)			
		0	30	90	150
1	Normal control (5% Tween 80)	86.16±3.13	107.16±2.15	117.66±1.87	86.33±2.41
2	Petroleumether (160mg/kg)	83.66±0.76	131.83±4.30^{c}	106.33±9.28	86±2.87
3	Chloroform (25mg/kg)	84.16±10.49	131±12.51^{c}	101±4.45	91.33±6.22
4	Ethylacetate (75mg/kg)	78.83±7.18	127.33±4.34	106.33±3.76	90.83±5.45
5	Butanol (100mg/kg)	75.16±1.51	110±1.98	87.33±1.40^{a}	69.5±1.52^{c}

Values are mean ± SEM, N=6: ^{a}P <0.001, ^{b}P <0.01, ^{c}P <0.05 vs. normal control.

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Successive extraction of the aqueous suspension of ehanolic extract with solvents resulted in petroleum ether, chloroform, ethylacetate and butanol fractions. The yields of different fractions as a % of ethanolic extract were as follows: Petroleum ether 53; chloroform 3.8; ethyl acetate 2.9; butanol 15.4; water (remaining), 24.9 (values are averages of 3 separate determinations). As shown in (Table 4) when different fractions were tested for their antihyperglycemic activity using glucose tolerance test in normoglycemic rats, the butanol fraction was found active at 15.4 mg/kg level. Pet ether fraction also showed activity to a lesser extent at 53 mg/kg level. The chloroform and ethylacetate fractions were inactive.

CONCLUSION

Ethanolic extract of *Boerhavia diffusa* root exhibited significant antihyperglycemic activities in streptozotocin induced rats. Extract showed improvement in parameters like body weight and lipid profile as well as regeneration of pancreas cells and so might be a value in diabetes treatment.

REFERENCES

[1] Torres RC, Batista MM, Pons AH, Silva AR, Cordeiro RSB, Martins MA, Carvalho AV. European J Pharmacol (2012).
[2] Rai H, Singh P, Singh M, Khan A, Chauhan SK, Thapliyal K, Ojha SK. Journal of Pharmacy Research 2011; 4.
[3] Frode T, Medeiros Y. J Ethnopharmacol 2008; 115**:** 173-183.
[4] Bhatia V, Kinja K, Bishnoi H, Savita S, Gnaneshwari D. Recent Research in Science and Technology 2011; 3(7): 04-07.
[5] Pari L, Satheesh MA. J Ethnopharmacol 2004; 91: 109-113.
[6] Babu V, Gangadevi T, Subramoniam A. Indian J Pharmacol 2003; 35: 290-296.
[7] Aileen JF King. British J Pharmacol 2012; 166: 877–894.
[8] Kumar, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, Krishan MRV. J Med Plants Res 2008; 2(9): 246-249.
[9] Nader R, Bachorik PS, Albers J, Lipids, Lipoprotiens, Apolipoprotiens. Tietz fundamentals of clinical chemistry. 5th Ed, Burtis, C.A & Ashwood, E.R (W.B. Saunders eds, Philadelphia USA), 2001; 463.
[10] Bucolo G, David H. Clin Chem 1973; 19: 476-482
[11] Lopez-Virella, H. F., et al. J Lipid Res 1970; 11: 583-595.