PHARMACOLOGICAL EVALUATION OF BAUHINIA PURPUREA AGAINST STREPTOZOTOCIN AND HIGH FAT DIET INDUCED DIABETIC RATS.

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Abstract

Background: Diabetes mellitus is one of the most prevalent conditions in the world andit is one of the top most cause of death in the United States of America, India, Europe and Asia. In India, the prevalence of diabetes mellitus particularly type 2 ispredominant when compared to the western population. Though anti-diabetic drugs play a major role in managing, treating and preventing diabetes mellitus, the adverse effects are known.

Objective: The objective of the present study is to evaluate the Bauhinia purpurea against streptozotocin and high fat diet induced diabetes in rats.

Methods: The animals were grouped into six groups (n=6) in each group. Normal control rats were administered normal saline (0.5 ml/kg orally by oral gavages) daily. Diabetic rats induced with a single dose of streptozotocin (40 mg/kg b. w., i. p.). Rats were administered with vanaspati + coconut oil (3:2) (0.5ml/kg. bwt) orally daily for 28 days to induce a high fat diet (HFD) and then diabetes induction was done. Diabetic rats were administered with HFD and co-treated with ethanolic extract of *Bahunia purpurea* (EEBP) (250 mg/kg b. w.) orally. Diabetic rats were administered with HFD and co-treated with ethanolic extract of *Bahunia purpurea* (500 mg/kg b. w.) orally daily. Diabetic rats were administered with HFD and co-treated with metformin (250 mg/kg b. w.) orally daily.

Results: The high dose of EEBP(500mg) treatment for diabetic and HFD rats showed ($p<0.001$) reduced levels of glucose values proving its anti-diabetic properties, the efficacy is similar to that of metformin treatment. The lower dose of EEBP (250mg) also decreased the glucose level from the diabetic range but the significance was less compared to the high dose of the test drug. The insulin and glycogen levels are decreased in high-fat diet rats. The effect of EEBP (250 mg/kg b w& 500 mg/kg b w respectively) in diabetic rats was evident from the results of insulin and glycogen values. Administration of a high dose of EEBP caused significant ($p<0.01$) elevation in liver glycogen levels. The EEBP 500 mg/kg significantly ($P<0.001$) reduced serum levels of total cholesterol, triglyceride, very low-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol while significantly increasing the high-density lipoprotein-cholesterol in STZ alone induced diabetic rats fed with high-fat dietless reduction compared to metformin treated rats. Additionally, 250 mg/kg of the extract significantly ($P<0.01$) reduced serum lipid profile and lipoproteins level. The AST and ALT activities was in normal range in controlgroup and the value was significantly ($p<0.001$) high in STZ induced and HFD+STZ group. The ALT and AST activities was controlled to normal in Metformin treated group. The HFD+STZ+EEBP (250 mg) group rats less significantly reduced these enzymes than HFD+STZ+EEBP (500mg) treatment

Conclusion: From the results, it can be seen that *Achyranthes aspera* has both anti-diabetic and anti-obesity properties.

Key words: Bauhinia purpurea, Streptozotocin, High fat diet, Anti diabetic

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1. Introduction:

Diabetes mellitus is one of the most prevalent conditions in the world andit is one of the top most cause of death in the United States of America, India, Europe and Asia. There are several complications associated with it likecardiovascular disease, retinopathy, nephropathy, wound infection and so on. More drugs and research data are being done to manage and treat thecondition[1]. The management of diabetes mellitus depends on the type. Human diabetes mellitus type 2 can be divided into various stages. One of which includes late or early diabetes mellitus type 2[2]. The common forms of management options include oral medications, administration of insulin via injections or medications, emphasis on lifestyle factors like exercise and diet. The secondary complications of diabetes mellitus are also managed by appropriate methods of treatment.[3] This is crucial because the constant exposure to a hyperglycaemic state leads to an increase in secondary complications which in turn is responsible for the majority of the mortality and morbidity.[4]

Some of the secondary issues that arise out of diabetes mellitus include wound healing impairment, its association with vascular conditions, inflammations, lipid abnormalities and so on. Understanding the importance ofthe complications that arise out of diabetes is of vital importance in terms of research and developing potential therapeutic targets.[5]

Prevention of diabetes mellitus type 2 is very much necessary, factors like diet and exercise play a crucial role. By incorporating regular physical activities and diet one can prevent the condition. It has been established that almost 60% of individuals with the diabetic condition are obese in nature. Apartfrom these, regular screening is necessary for individuals who are at higher risk.A good example of it would be a family history of the same[6]. In India, the prevalence of diabetes mellitus particularly type 2 ispredominant when compared to the western population. It has also been foundthat the complication of microvascular and macrovascular are high in Indian populations which could be due to genetic factors associated with ethnicity[7]. Antidiabetic activity of leaves of ethanolic extract of Bauhinia purpurea against STZ and high fat diet is unexplored. The present study is undertaken to evaluate the antidiabetic activity of Bauhinia purpurea in rats.

2. Material and Methods

2.1. Collection and authentication of plant

Bauhinia purpurea plant leaves were collected from forest region of Tirupati, India. The herbarium was prepared and authenticated by Dr. Madhava chetty, Sri Venkateshwara University, Tirupati. The authentication voucher specimen number is 0611.

2.2. Extraction procedure:

Preparation of *Bauhinia purpurea* leaf extract

The leaves were initially separated from the main parts of body and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. A total of 150g of leaf air dried powder was weighed and was placed in 500ml of organic solvents[ethanol]in a borosil screw cap bottle/container. The container is then closed and kept for atleast 3 days. The content is stirred periodically and if placed inside a bottle it should be shaken time to time to ensure complete extraction and then it was filtered with the help of muslin cloth and then kept in a rotary evaporator. Subsequently, the micelle is separated from the menstrum by the evaporation in an oven on top of water bath, extract was filtered and dried in china dish.

2. 3. Animals

Healthy adult female albino rats (120-200gram body weight) were randomly assigned to 5 groups, each containing 6 animals in polypropylene cages layered with husk and maintained in a controlled room at a temperature (22 ± 3°C) and light (12 hours light/dark cycle). Animals were allowed free access to water and standard pellet diet. Animals were cared in accordance with the "Guide for the care and use of laboratory animals" and study was conducted in accordance with CPCSEA. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (No: CPCSEA/II/ 08/RIPER/11).

2.4.Experimental Design

The animals were grouped into six groups (n=6) in each group. Group 1: Normal control rats were administered normal saline (0.5 ml/kg orally by oral gavages) daily. Group 2: Diabetic rats induced with a single dose of streptozotocin (40 mg/kg b. w., i. p.). Group 3: Rats were administered with

vanaspati + coconut oil (3:2) (0.5ml/kg. bwt) orally daily for 28 days to induce a high fat diet (HFD) and then diabetes induction was done. Group 4: Diabetic rats were administered with HFD and co-treated with ethanolic extract of *Bahunia purpurea* (EEBP) (250 mg/kg b. w.) orally. Group 5: Diabetic rats were administered with HFD and co-treated with ethanolic extract of *Bahunia purpurea* (500 mg/kg b. w.) orally daily. Group 6: Diabetic rats were administered with HFD and co-treated with metformin (250 mg/kg b. w.) orally daily.

Statistical Analysis:

The results were expressed as mean ± SEM and statistically analyzed by using one way ANOVA followed by dunnettes multiple comparison test. $P<0.05$ is considered to be significant.

3. Results and Discussion

Initial body weight was normal in all the groups of rats. Thereafter, the difference increased throughout the study in high fat dietinduced rats. The highest body weight value above 300 g was above in diabetesand diabetic+HFD rats whereas 175g was noted in normal rats. The weight reduction in EEBP (500mg) treated rats was similar to that of metformin-treated rats. The delayed bodyweight improvement was noted in EEBP (250mg) dose group. it can be seen that the glucose levels are the highest in diabetic rats fed with a high-fat diet while it was less high in diabetic rats compared with normal the control group. There are ($p<0.001$) significant differences in the levels of glucose between each group. The STZ and STZ+HFD groups glucose values above 200 mg/dl. The high dose of EEBP (500mg) treatment for diabetic and HFD rats showed ($p<0.001$) reduced levelsof glucose values proving its anti-diabetic properties, the efficacy is similar to that of metformin treatment. The lower dose of EEBP (250mg) also decreased the glucose level from the diabetic range but the significance was less compared to the high dose of the test drug. The insulin and glycogen levels are decreased in high-fat diet rats induced diabetes followed by diabetic rats. The results were comparable to that of metformin, a standard drug. The effect of EEBP (250 mg/kg b w& 500 mg/kg b w respectively) in diabetic rats was evident from the results of insulin and glycogen values. Animals treated with the standard drug also showa significant reduction in their blood glucose and HbA1C level compared to Group II ($p<0.05$) and Group III ($p<0.001$). Administration of a high dose of HEAA caused significant ($p<0.05$) elevation in liver glycogen levels the metformin also significantly decreased the HbA1C and glycogen levelsin diabetic rats. The HEAA 500 mg/kg significantly ($P<0.001$) reduced serum levels of total cholesterol, triglyceride, very low-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol while significantly increasing the high-density lipoprotein-cholesterol in STZ alone induced diabetic rats fed with high-fat diet less reduction compared to metformin treated rats. Additionally, 250 mg/kg of the extract significantly ($P<0.01$) reduced serum lipid profile and lipoproteins level. Furthermore, the AST and the ALT activity were also analysed across the different groups. The AST and ALT activities was in normal range in controlgroup and the value was significantly ($p<0.001$) high in STZ induced and HFD+STZ group. The ALT and AST activities was controlled to normal in Metformin treated group. The HFD+STZ+HEAA (250 mg) group rats less significantly reduced these enzymes than HFD+STZ+HEAA (500mg) treatmentrats.

4. Conclusion

From the results, it can be seen that *Bauhinia purpurea* has both anti-diabetic and anti-obesity properties. Significant reductions in glucose and lipid content were found after the administration of *Bauhinia purpurea* thereby proving its therapeutic properties.

5. References

1. Adeyi, A. O, Idowu, B. A, Mafiana, C. F, Oluwalana, S. A, Ajayi, O. L, and Akinloye, O. A. Rat model of food-induced non-obese-type 2 diabetes mellitus: comparative pathophysiology and histopathology. International Journal of Physiology, Pathophysiology and Pharmacology. 2012,4(1), 51.
2. Adil, M, Khan, R. A, Kalam, A, Venkata, S. K, Kandhare, A. D, Ghosh, P and Sharma, M. Effect of anti-diabetic drugs on bone metabolism: Evidence from preclinical and clinical studies. Pharmacological Reports. 2017,69(6), 1328–1340.
3. Alam, F, Islam, M. A, Khalil, M. I, and Gan, S. H. (2016). Metabolic Control of Type 2 Diabetes by Targeting the GLUT4 Glucose Transporter: Intervention Approaches. Current Pharmaceutical Design. 2016, 22(20), 3034–3049.
4. Alam, M. T, Karim, M. M, and Khan, S. N. Antibacterial Activity of Different Organic Extracts of Achyranthes aspera and Cassia Alata. Journal of Scientific Research. 2009,1 (2), 393–398.
5. Arınç, E, Arslan, Ş, and Adalı, O. Differential effects of diabetes on CYP2E1 and CYP2B4

proteins and associated drug metabolizing enzyme activities in rabbit liver. Archives of Toxicology. 2005, 79(8), 427–433.

6. Armstrong, J. A, Cash, N, Soares, P. M. G, Souza, M. H. L. P, Sutton, R, and Criddle, D. N. Oxidative stress in acute pancreatitis: Lost in translation? Free Radical Research. 2013,47 (11), 917–933.

Table 1: Effect of Bauhinia purpurea extract extracts on Physiological parameters

Group	Initial body weight(g)	Final body weight(g)	Food intake (g/day)	Water intake (ml/day)
Group 1	182.9±4.06	224.9±5.19	18.9±0.15	21.1±0.92
Group 2	176.7±1.68^{ns}	282.8±2.52***	23.8±0.44***	18.5±0.13*
Group 3	176.9±0.76^{ns}	180.1±2.83$^{###}$	19.3±0.40$^{###}$	19.1±0.18^{ns}
Group 4	176.4±4.60^{ns}	196.4±3.32$^{###}$	21.4±0.28$^{##}$	18.0±0.18^{ns}
Group 5	170.8±2.35^{ns}	234.8±7.21$^{###}$	20.1±0.84$^{##}$	18.7±0.49^{ns}
Group 6	174.5±0.99	177.7±0.96$^{###}$	18.7±0.36$^{###}$	17.3±0.32^{ns}

All values are expressed mean± SEM (N=6)., ***P<0.001 compared with normal control, *P<0.05 compared with normal control, $^{###}$P<0.001 compared with group 2, ns – non significant compared with Group 2.

Table 2: Effect of Bauhinia purpurea extract on Blood glucose

Group	2nd week	4th week	8th week
Group 1	89.5 ± 1.48	98.7 ± 5.66	91.25± 6.02
Group 2	178.8± 1.86**	179 ± 2.91***	276.7 ± 7.82***
Group 3	140.5±3.92 ^{ns}	141 ± 2.44$^{###}$	129.1 ± 4.06$^{###}$
Group 4	133.8± 1.68 $^{###}$	131 ± 1.94$^{###}$	86.74 ± 6.14$^{###}$
Group 5	127 ± 1.34$^{###}$	104 ± 8.12$^{###}$	81.7 ± 1.81$^{###}$
Group 6	138.7 ± 2.03$^{###}$	144 ± 1.40$^{###}$	115.3 ±5.34$^{###}$

All values are expressed mean±SEM (N=6).
**P<0.01 compared with normal control
####P<0.0001 compared with group 2
ns = non-significant compared with group 2
##P<0.01 compared with group 2

Table 3: Effect of Bauhinia purpurea on HbA1C, Plasma Insulin.

Group	HbA1C	Plasma Insulin (µIU/ml)
NC	2.34±0.08	14.0 ± 0.40
FD	9.05±0.19***	4.40 ± 0.24***
FD + HELA	4.07±0.21$^{###}$	12.3 ± 0.25$^{###}$
FD + EAELA	5.21±0.21$^{###}$	11.4 ± 0.46$^{###}$
FD + Metformin	3.38±0.17$^{###}$	12.8 ± 0.46$^{###}$
FD + Simvastatin	4.58±0.40$^{##}$	10.4 ± 0.31$^{###}$

All values are expressed mean ±SEM (N=6).
**P<0.01 compared with normal control
####P<0.0001 compared with fructose diet
###P<0.001 compared with fructose diet
##P<0.01 compared with fructose diet

Table 4: Effect of Lupinus Bauhinia Purpurea on Lipid Profile

Group	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Group 1	69.2±0.73	90.1±1.15	38.7±1.61	14.9±0.66	28.2±0.27
Group 2	160±2.25***	179.5±5.29***	143.9±1.66***	38.0±0.38***	17.2±0.66***
Group 3	70.9±0.74$^{###}$	116.1±4.62 $^{###}$	47.0±3.12$^{###}$	17.8±0.24 $^{###}$	25.8±0.89$^{###}$
Group 4	84.9±1.48 $^{###}$	139.3±2.12 $^{###}$	74.0±1.80 $^{###}$	25.4±1.52 $^{###}$	19.15±0.59^{ns}
Group 5	125±5.18$^{##}$	181.7±4.40^{ns}	139.8±1.50^{ns}	34.4±1.08^{ns}	17.5±0.52^{ns}
Group 6	70.8±1.07$^{###}$	84.8±0.59$^{###}$	43.7±1.20$^{###}$	15.5±0.59$^{###}$	27.9±0.52$^{###}$

All values are expressed mean±SEM (N=6).
**P<0.01 compared with normal control
$^{####}$P<0.0001 compared with fructose diet
ns = non-significant compared with fructose diet

###P<0.001 compared with fructose diet
##P<0.01 compared with fructose diet

Table 5: Effect of Bauhinia purpurea extract on Liver function markers

Group	SGPT (U/l)	SGOT (U/l)
Group 1	71.1±2.03	80.7±1.21
Group 2	212.8±2.84***	256.0±13.55***
Group 3	115.9±5.77###	108.2±2.86###
Group 4	133.6±3.16###	158.9±0.95###
Group 5	141.3±1.66###	185.9±1.13###
Group 6	109.2±0.94###	109.5±1.93###

All values are expressed mean±SEM (N=6). **P<0.01 compared with normal control, ###P<0.001 compared with fructose diet, ###P<0.001 compared with fructose diet, ##P<0.01 compared with fructose diet.