

Evaluation of Anti Hyperglycemic Studies on *Hypericum hookerianum* in Streptozotocin(STZ) and Nicotinamide Induced Diabetic Rats

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ABSTRACT

The aim of this study was investigate antihyperglycemic effect of ethyl acetate extracts of leaves and stem of *Hypericum hookerianum* (HH) as in streptozotocin and nicotinamide induced diabetic rats. Male Wistar rats were administrated with leaf (HHL) and stem(HHS) extracts (200 mg/kg)of *Hypericum hookerianum* orally for 28days and blood glucose was measured once in week about 4weeks.The HHL extract showed more reduction in blood glucose level by 49 % on the 4th week of treatment when compared to controlled STZ group. At the end of the study significant reduction in lipid profile such as Triglycerides, HDL, Total Cholesterol, along with urea, uric acid and creatinine were found to be reduced in treated group. The experimental data revealed that the HHL extract posses significant antihyperglycemic effects than HHS extracts.

Keywords: *Hypericum hookerianum*, Streptozotocin, Nicotinamide, Anti-hyperglycemic

INTRODUCTION

Diabetes Mellitus is a group of heterogeneous, hormonal and metabolic disorders characterized by elevated blood glucose levels called hyperglycemia. This may be caused by decreased insulin secretion, insulin action or both, with disturbances of carbohydrate, fat and protein metabolism¹. Recent survey reported that more than 100 million people are affected with Diabetes worldwide. Further it is also getting raised in number with increasing population every year. It is being predicted that the number of diabetes patients would reach 400 million around the world before 2030. The world largest diabetic population has been reported in India, and was estimated to reach 200 million in the year of 2025 in India^{2,3}.

The medicinal community has a challenge to search an alternative novel compounds from natural source. In Indian system of medicine, many herbs have long been introduced and in practice for the treatment of diabetes. The WHO also recommended and encouraged this practice to recognize the traditional system of medicine^{4,5}.

The plant *Hypericum hookerianum* is small shrubs distributed in Himalayan region and in Nilgiri district, Tamilnadu approximately 400 species of *Hypericum* were found worldwide. In India more than 20 species have been reported (Robson 1968,1977). The plant has reported for antidepressant, antiulcer, antibacterial, antiviral, antioxidant and wound healing activity.

The principle constituents reported from this plant includes naphthodianthrones, hypericin, hyperforin, pseudohypericin, flavanoids include quercetin, quercitrin, rutin and essential oils

The hyperforin is one among compound in flavanoid group and it posses antioxidant, antidiabetic, anticancer potentials. Recent survey reported that cytokine induced beta cell injury has improved with administration of hyperforin, hence beta cell loss could be prevented in diabetic condition by *Hypericum* species⁶⁻⁹.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Co., all other chemicals were purchased from SD fine chemicals and the reagent used was of analytical grade.

Collection and authentication of plant material

The leaves and stems of *Hypericum hookerianum* (HH) Wight&Arn was collected in and around of Nilgiri district, South India, Tamilnadu, India and the plant was authenticated by Dr.Rajan, Field Botanist, Bandishola, Ooty, Tamilnadu and the specimen voucher (KMCHCP/POG/213-2011)was preserved in the department for further references.

Preparation of Extracts

The shade dried leaves and stems were made into coarse powder and extracted respectively with petroleum ether and ethyl acetate using Soxhlet apparatus for about 48 hrs. The ethyl acetate extract was concentrated by rotary evaporator and stored in desiccators until use.

Procurement of experimental animals

Adult male wistar albino rats of weighing 150-200 g were used in the present study. All the animals were procured from animal house, KMCH College of Pharmacy,

Coimbatore, Tamilnadu. All the rats were maintained at 24-28°C with standard laboratory feed (Lipton India Ltd) and water *ad libitum*.

Induction of Diabetes

Experimental type 2 diabetes mellitus was developed in adult rats by administering streptozotocin (STZ) and nicotinamide (NA) in 0.1 M citrate buffer, pH 4.5. Overnight fasted rats were administered with single dose streptozotocin (60 mg/kg/i.p), 15 min after the administration of (120mg/kg/i.p) nicotinamide. After injection rats were allowed free access to feed and provide with 5% glucose solution to drink overnight to control the hypoglycemic shock. The development of diabetes was confirmed after 48h from fasted rats. The rats having fasting blood glucose level more than 250mg/dL were selected and grouped for the studies¹⁰⁻¹².

Experimental protocol

The study was conducted on 30 rats, these were made into 5 groups each group consisted of 6 animals.

Group I: Non diabetic control (Only vehicle)

Group II: Diabetic control rats treated with single dose of STZ 60mg/kg and Nicotinamide (NA) 120mg/kg, i.p.

Group III: Diabetic rats treated with standard Metformin 40mg/kg

Group IV: Diabetes rats treated with ethyl acetate leaf extract of HH 200mg/kg

Group V: Diabetes rats treated with ethyl acetate stem extract of HH 200mg/kg

All the rats were received the treatment by the above schedule for about 28 days. Blood samples were collected from tail vein of rats on 1, 7, 14, 21 and 28th day and fasted blood glucose level was determined by using one touch Accu-chek electronic glucometer using glucose strips. (Lifescan, Johnson and Johnson Ltd)¹³⁻¹⁴.

At the end of the study all the animals were anesthetized using ethyl ether and blood was collected by retro orbital puncture and biochemical parameters like triglycerides (TG), cholesterol (CH), high density lipoprotein (HDL), urea and uric acid values were estimated from the serum¹⁵.

Statistical analysis

The data are expressed as mean \pm standard error (S.E.) and the treated groups were compared to control and standard groups. All the results were tabulated by ANOVA followed by Dunnett's *t* - test. *p* value <0.001 was considered to be statistically significant. (Dixon and Jennrich, 1990).

RESULTS

Streptozotocin induced hyperglycemic effect has described as a useful experimental model to study the diabetic activity. The fasting blood glucose levels of all experimental rats were measured at 1, 7, 14, 21 & 28 days respectively. There was a significant elevation in blood glucose level initially found from the entire group except vehicle treated group. The standard metformin (40mg/kg) treated group has its expected glucose lowering effect in diabetic rats.

Effect of HHL and HHS on Body weight

The body weight of the experimental rats were weighed on 0, 7, 14, 21 and 28 days. The body weight of the streptozotocin treated control group was found to be reduced significantly (*p*<0.001) compared to normal group. The treatment of standard drug metformin (40mg/kg) resulted in a significant (*p*<0.001) recovery of body weight in standard group. Similarly, the treatment of tested groups crude extracts of HHL and HHS possess significant (*p*<0.001) recovery of body weight.

Effect of HHL and HHS on Blood Glucose levels

The fasted blood glucose level of the experimental rats were measured on 0, 7, 14, 21 and 28 days. The administration of streptozotocin and nicotinamide resulted in significant hyperglycemia in control, standard and test groups. The blood glucose levels were significantly reduced (*p*<0.001) from 272.0 ± 1.86 on to 99.0 ± 1.36 in standard group treated with 40mg/kg of metformin. The test extract of HHL also significantly (*p*<0.001) reduced the blood glucose levels from 271.3 ± 1.60 to 127.2 ± 1.40 . The test extract of HHS also significantly (*p*<0.001) reduced the blood glucose levels from 269.7 ± 2.10 to 138.5 ± 1.11 at dose of 200mg/kg respectively. The investigation of reduction in blood glucose levels were found 60%, 49% and 44% between standard, HHL and HHS respectively when compared with control group.

Effect of HHL and HHS on Plasma Lipid Contents

The TG, HDL and TC of the experimental rats were measured on 0, 7, 14, 21 and 28 days. The TG and TC levels were increased in streptozotocin treated groups and the HDL level was decreased streptozotocin treated groups. The standard group treated with 100mg/kg of metformin significantly (*p*<0.001) restored the levels of TG, HDL and TC. The test groups treated with HHL and HHS also significantly (*p*<0.001) restored the levels of TG, HDL and TC.

The level of urea, uric acid and creatinine were increased in animals treated with streptozotocin. The treatment of animals in standard group with 100mg/kg of metformin significantly (*p*<0.001) restored the increased levels of urea, uric acid and creatinine to normal levels. The treatment of test animals with extracts of HHL and HHS significantly (*p*<0.001) restores the levels of urea, uric acid and creatinine.

DISCUSSION

The present study was investigate the anti hyperglycemic activity from ethyl acetate extracts of leaves and stems of HH against Streptozotocin (STZ) and nicotinamide (NA) induced type II diabetic rats. The STZ and nicotinamide induced diabetic model is one of the common screening method to represent type II diabetes mellitus. NA and STZ produces stable and moderate hyperglycemia method for type 2 diabetic studies. Streptozotocin alone it selectively destroys the pancreatic β cells but NA partially protects the β cells against the STZ mediated cytotoxic damages. NA was found to preserve the intracellular pool of NAD either by acting as a precursor of NAD or by inhibiting the activity of poly (ADP-ribose) synthesis which is an NAD consuming enzyme activate by STZ

Table 1: Shows the effect of ethyl acetate extracts of leaves and stems of *Hypericum hookerianum* Wight & Arn on the body weight of animals.

S. No	Treatment	Change in body weight (gm)				
		Initial body weight	1 st week	2 nd week	3 rd week	4 th week
1	Vehicle	158.8±2.62	165.0±2.22	172.2±2.23	176.8±3.25	182.2±3.68
2	Only STZ	159.5±2.76	148.2±2.08	141.0±1.34	137.0±3.78	132.5±2.60
3	STZ + STD Metformin (40mg/kg)	157.2±3.17	163.2**±2.15	166.8±2.97***	167.5±4.04***	168.2±1.83***
4	STZ + H.H leaf extract (200mg/kg)	159.2±3.66	161.7**±1.64	164.0±2.06***	164.8±2.89***	165.5±4.49***
5	STZ + H.H stem extract (200mg/kg)	157.8±2.8	162.7±1.92**	163.7±2.51***	164.0±2.89**	165.7±2.30***

Data was expressed as mean ±SEM. (n = 6 animals in each group). Values are statistically ***P<0.001, **P<0.01,*P<0.05,ns = non significant,more significant at P<0.001.

Table 2: Shows the effect of ethylacetate extracts of leaves and stems of *Hypericum hookerianum* Wight & Arn., on the fasting blood glucose level in control and experimental animals

S. No	Treatment	Change in Blood Glucose level (mg/dl)				
		Day 1	Day 7	Day 14	Day 21	Day 28
1	Normal	90.17±3.9	88.833±2.7	90.17±1.4	89.83±1.07	89.33±0.88
2	Control Only STZ	262.2±1.66	271.33±1.17	261.5±1.58	258.7±1.60	247.7±1.24
3	STZ+ Metformin(100 mg/kg)	272.7±1.86	173.70±3.11***	148.7±1.17***	115.7±1.85***	99.10±1.36***
4	STZ + HH Leaf extract 200 mg/kg	271.3±1.60	218.30±2.70***	182.7±1.40***	135.8±1.97***	127.2±1.40***
5	STZ + HH Stem extract 200 mg/kg	269.7±2.1	233.0±1.98***	204.7±1.70***	161.3±2.61***	138.50±1.11**

Data expressed as mean ±SEM. (n = 6). Values are statistically significant at P<0.001.*P<0.05,**P<0.01,***P<0.001(vs Control) &^aP<0.001(vs Normal).

Table 3: Shows the effect of ethylacetate extracts of leaves and stems of *Hypericum hookerianum* Wight & Arn., on serum biological parameters in control and experimental animals.

Parameters	Normal	Control	STZ + Metformin (40mg/kg)	STZ + HHL extract (200mg/kg)	STZ + HHS extract (200mg/kg)
TG	130.7±0.88	167.8±0.94	133.7±1.43***	159.3±1.43***	158.2±1.10***
HDL	65.0±0.57	43.50±0.56 ^a	59.33±0.49***	48.00±0.57***	50.00±0.36***
Total Cholesterol	104.2±1.04	145.3±1.14	97.67±1.10***	115.0±1.0***	116.7±1.05***
UREA	25.67±0.66	70.10±0.57	24.0±0.73***	42.83±1.16***	45.83±0.79***
URIC ACID	2.6±0.18	6.83±0.30	2.75±0.10***	5.60±0.08***	5.65±0.042***
Creatinine	0.94±0.10	2.20±0.06	1.21±0.01*	1.77±0.04*	1.70±0.05*

The effect of ethylacetate leaf and stem extracts of *Hypericum Hookerianum* Wight & Arn., on serum biochemical parameters are expressed as mean ± S.E.M; (n=6), ^aP<0.001 (vs Normal), ***P<0.001(vs Control).

Induction of diabetes with STZ is associated with characteristic loss of body weight and pancreatic β -cell necrosis will occur it leads to persistent hyperglycemia. Deficiency of insulin results in a catabolic state that affects glucose, fat, and protein metabolism. This abolishes

assimilation of glucose in the muscle and adipose tissue, so gluconeogenesis attributed to body weight loss in DM. Sulfonyl urea such as glibenclamide is the most widely used drugs for the treatment of type-II diabetes by stimulating insulin secretion.

In the present study diabetic rats were observed significant reduction of elevated blood glucose level for the extracts treated group from the 2nd week. Generally the anti diabetic plants produce its activity by inhibition of carbohydrate metabolizing enzymes, enhancement of insulin sensitivity, and regeneration of pancreatic islets β -cells or the enhancement of insulin secretion. The ethyl extract of *HH* leaf and stem possibly act via any one of the above mechanism. The ethylacetate extracts of *HHL* showed significant lowering blood glucose level than that of the *HHS*.

In STZ induced diabetic rats elevated cholesterol, TG levels and decreased HDL level was observed. This is because the excess fatty acids in the serum of diabetic rats are converted to phospholipids and cholesterol in the liver. Then phospholipids and cholesterol formed along with triglycerides in the liver are discharged into blood in the form of lipoproteins. In present study, a significant increased level of cholesterol and TG as well as marked decrease in serum HDL cholesterol level was noticed in diabetic rats. After treatment with extracts, decreased level of cholesterol and TG as well as increased level of HDL in diabetic treated rats were noticed. The above effects might be advantageous in preventing diabetic complications like coronary heart disease and atherosclerosis also for improving lipid metabolism in diabetic condition.

CONCLUSION

The ethyl acetate extract of *Hypericum hookerianum* is beneficial in controlling the blood glucose level by improve the lipid metabolism. The flavonoid compounds present in this extract may be involved for control of DM and overall *HHL* extract possess significant anti-diabetic activity than *HHS* extract.

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