ANTIDIABETIC EFFECT OF *HOLARRHENA ANTIDYSENTERICA* SEEDS ON STREPTOZOTOCIN INDUCED DIABETIC RATS.

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Summary

This study was undertaken to evaluate the antidiabetic effect of Petroleum ether (PEHAD) and aqueous (AEHAD) extracts of *Holarrhena antidysenterica* seeds in normal (Normoglycemic) and in streptozotocin (STZ) induced diabetic rats. On chronic treatment, both petroleum and aqueous extract has showed significant decrease in blood glucose level (BGL), serum cholesterol, triglyceride levels and at the same time markedly increased liver glycogen, thus proving the potentiating property of *Holarrhena antidysenteric* as antidiabetic drug.

Keywords: *Holarrhena antidysenterica*, Diabetes, Streptozotocin

Introduction

Diabetes mellitus is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia).[1] Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or faliure of various organs like eyes, kidneys, nerves, heart and blood vessels. Diabetes is possibly the world's largest growing metabolic disorders, and as the knowledge on the heterogencity of this disorder is advanced, the need for more appropriate therapy increases. A no. of allopathic drugs used for there antidiabetic effect like tolbutamide, metformin, phenformin, acarbose have danger of drug interaction, adverse effect etc.[2]

Tradittional plant medicines are used throughout the world for diabetes. There are many medicinal plants known to be used in the treatment of diabetes and a number of plants have been screened positive for their antidiabetic effect. Most of these plants were found to belong to the chemical groups glycosides, alkoloids and flavonoids.[3] *Holarrhena antidysenterica* is a typical Indian medicinal plant and is rich of flavonoids, alkaloids etc.[4,5] The bark and seeds are used to treat amoebic dysentery, diarrhea, asthma, bronchopneumonia and malaria.[6] Decoction of seeds was recommended in cases of diabetes.[7] However, the search for an ideal antidiabetic agent continues and therefore, this plant has been taken to explore its potentiating effect on diabetes.

Materials and Methods

Collection of plant

The seeds of *Holarrhena antidysenterica* were procured commercially from "Amruth Kashri", Bangalore. The seeds were authenticated by Dr. Shiddamallayya N, Regional Research Institute (Ay.) Govt. Central Pharmacy Annexe, Ashoka Pilar, Bangalore-11.

Preparation of aqueous and petrolium extract of Holarrhena antidysenterica

The PEHAD and AEHAD extract were prepared by Soxhlet extraction of 500 g seed powder in 1000 ml of petroleum ether and distilled water according to their increasing polarity. The extract was concentrated dried in vacuum (yield 8.8%) and residue stored in refrigerator at 2-8°C for used in subsequent experiments.

Experimental animals

Male wistar rats weighing about 200-250 g were used for the study. All animals were kept and maintained under laboratory conditions of temperature (22 ± 2°C) and 12 h day:12 h night cycle as per guidelines (CPCSEA, 2003). Animals were allowed free access to food (standard pellet diet) and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee of Acharya & B.M Reddy college of Pharmacy, Bangalore, India.

Induction of Diabetes

After 1 week acclimatization, rats were fasted overnight. Diabetes was induced by a single intravenous (i.v) injection of streptozotocin 35 mg/kg dissolved in 0.1 M Citrate buffer (pH 4.5). After 48 hours of STZ injection, fasting BGL has been checked and rats having BGL >250 mg/dl were considered as diabetic and taken into study.

Experimental design and treatment schedule

The diabetic rats were divided randomly into 5 groups consisting of 10 animals each. The group (I) was normal control and received an equal volume of physiological saline and 2% gum acacia.

The group (II) of STZ induced rats received an equal volume of physiological saline and 2% gum acacia was taken as diabetic control. The group (III) of STZ induced rats received glibenclamide (10 mg/kg, p.o) was taken as reference standard. The group (IV) and (V) of STZ induced rats received petroleum ether (250 mg/kg) and aqueous (250 mg/kg) extract of *Holarrhena antidysenterica* for 18 days served as treatment groups.

Statistical Analysis

Results are expressed as the mean ± S.E. and statistical significance between treated and diabetic control group was analyzed by one-way ANOVA followed by Student's *t*-test and $P < 0.05$ was considered significant.

Results

Significant weight loss was observed in diabetic control group than normal control group. The PEHAD, AEHAD and glibenclamide shown an increase in body weight compared to diabetic control group (Table 1). Administration of PEHAD, AEHAD and glibenclamide led to significantly decreased in blood glucose levels when compared with diabetic control (Table 2).

Significant difference was also observed in liver glycogen level and total protein level estimated in diabetic rats (Table 3). PEHAD and AEHAD has shown significant decrease in serum triglyceride and cholesterol levels when compared with diabetic control group (Table 4).

Table-1

Group of Animals	Body Weight of Different Group (g)			
	0^{th} Day	6^{th} Day	12^{th} Day	18^{th} Day
Normal Control	229.50 ± 2.53	251.00 ± 6.84	253.00 ± 2.58	259.00 ± 9.79
Diabetic Control	190.67 ± 3.72	175.12 ± 2.14	163.50 ± 0.99	129.00 ± 1.59
Diabetic+ Glibenclamide	193.17 ± 2.20	205.00 ± 2.29*	209.68 ± 3.98*	212.78 ± 2.08*
Diabetic+ PEHAD	198.83 ± 1.30	198.00 ± 4.22*	201.35 ± 4.23*	208.67 ± 3.46*
Diabetic+ AEHAD	189.12 ±3.25	192.83 ± 0.31	200.5 ± 0.43*	205.33 ± 0.80*

Values are expressed as mean ± S.E.M (n=6) * *P* < 0.05 vs. Diabetic control.

Table-2

Group of Animals	Blood glucose level (mg/dl)			
	0^{th} Day	6^{th} Day	12^{th} Day	18^{th} Day
Normal Control	107.67 ± 3.77	107.67 ± 3.19	106.50 ± 3.98	108.50 ± 4.32
Diabetic Control	320.50 ± 5.37	321.33 ± 5.51	321.16 ± 5.91	320.33 ± 4.88
Diabetic+ Glibenclamide	344.00 ± 1.78	327.16 ± 8.86	258.33 ± 12.81*	136.33 ± 3.26*
Diabetic+ PEHAD	328.00 ± 1.00	311.67± 2.30	259.00 ± 1.15*	169.83± 0.60*
Diabetic+ AEHAD	329.83 ± 0.79	315.92± 1.07	267.00 ± 0.89*	171.50± 2.51*

Values are expressed as mean ± S.E. (n=6) * *P* < 0.05 vs. Diabetic control.

Table-3

Group of Animals	Liver glycogen (mg/ of tissue)	Total protein (g/dl)
Normal Control	35.87 ±1.10	5.86 ± 0.19
Diabetic Control	23.31 ± 0.85	7.41 ± 0.42
Diabetic+ Glibenclamide	36.11 ± 0.98*	5.91 ± 0.20*
Diabetic+ PEHAD	46.33 ± 2.28*	6.38 ± 0.19*
Diabetic+ AEHAD	41.33 ± 2.60*	6.97± 0.37*

Values are expressed as mean ± S.E. (n=6) * *P* < 0.05 vs. Diabetic control.

Table-4

Group of Animals	Serum triglyceride (mg/dl)	Serum cholesterol (mg/dl)
Normal Control	139.30 ± 1.36	51.96 ± 1.77
Diabetic Control	187.27 ± 3.13	84.25 ± 1.44
Diabetic+ Glibenclamide	141.77 ± 1.88*	50.80 ± 0.22*
Diabetic+ PEHAD	137.17 ± 3.26*	56.33 ± 2.89*
Diabetic+ AEHAD	144.67 ± 2.46*	59.33 ±3.27*

Values are expressed as mean ± S.E. (n=6) * *P* < 0.05 vs. Diabetic control.

Discussion

In the present study, STZ produced high blood glucose levels in untreated STZ induced diabetic rats which may be due to selective destruction of pancreatic *beta* cells.[8] In the present study, administration of PEHAD and AEHAD for a period of 18 days to diabetic rats showed a significant decreased in the blood glucose levels. The possible mechanism by which petroleum and aqueous extract showed its glucose lowering activity may be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from the existing *beta* cells or by its release from the bound insulin or by proliferation of beta cells.[9] Significant increase in body weight has been observed in treated STZ diabetic rats. This could be the result of improved glycaemic control[10] provided by PEHAD and AEHAD. Triglyceride and cholesterol levels are found high in diabetic rats when compared with treatment group. These high levels have found in the diabetic rats as a result of lack of insulin which activates the lipase enzymes, hydrolyzing the stored triglycerides and releasing large amount of fatty acids and glycerol into the circulating blood. Consequently, the excess of fatty acids in the plasma may promote the hepatic conversation of fatty acids into phospholipids and cholesterol, the main products of lipid

metabolism.[11] At the same time glycogen, cortisol, catecholamine and growth hormones enhance lipolysis.[12,13]

Protein synthesis has been reduced due to lack of insulin in diabetic rats[14,]. Liver glycogen was Significantly reduced in diabetic rats, which results in inactivation of glycogen synthestase system. Treatment with petroleum and aqueous extract showed a significant decreased in the serum cholesterol, serum triglycerides indicating an increase in insulin level. Also AEHAD and PEHAD showed significant increase in total protein and liver glycogen levels. Thus representing the antidiabetic action of *Holarrhena antidysenterica which* may be due to improvement of glycogenesis.

Conclusions

In the present study, AEHAD and PEHAD has been taken in the study. AEHAD and PEHAD has showed significant effect on the blood glucose level, liver glycogen, serum lipids and body weight. These effect represent the antidiabetic potency of *Holarrhena antidysenterica*. Further studies are necessary to ascertain the use of *Holarrhena antidysenterica* seeds in diabetic complications.

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References

1. Rang HP, Dale MM., Ritter JM, Moore PK. Essentials of Pharmacology, 5th ed. Edinburgh, Churchill Livingstone, 1999:385.
2. Kamaeswara Rao B, Giri R, Kesavulu MM, Apparao Ch. Effect of oral administration of bark of Pterocarpus santalinus L. on blood glucose level in experimental animals. J. Ethnopharmacol 2001; 74:69–74.
3. Okonta JM, Aguwa CN. Evaluation of Hypoglycemic Activity of Glycosides and Alkaloids Extracts of Picralima nitida Stapf (Apocynaceae) Seed. Int. J. Pharmacol 2007; 3:505-509.
4. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. India, Nirali Prakashani, 2006:516-517.
5. K.R. Khandelwal.Practical Pharmacognosy. Nirali Prakashani, India.
6. Kumar A, Ali M. A new steroidal alkaloid from the seeds of Holarrhena antidysenterica. Fitoterapia 2000; 71:101-104.
7. Ghose SC., Drugs of Hindusthan. Hanhemann Publishing CO Pvt. Ltd, Calcutta.
8. Aldo A. Rossini, Arthur A. Like, William L. Chick, Michael C. Appel. Studies of streptozotocin-induced insulitis and diabetes. Proc Natl Sci USA 1977; 74:2485-2489.
9. Annie Shirwaikar, Rajendran K, Rakesh Barik. Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol 2006; 107**:**285-290.

10. Momo Claudia EN, Oben Julius E, Tazoo Dagobert, Dongo Eienne, Afr. J. Trad. CAM 2006; 3:36-43.
11. Daamasceno DC, Volpato GT, I. De Mattos Paranhos Calderon, Aguilar R, Cunha Rudge MV. Effect of Bauhinia forficata extract in diabetic pregnant rats maternal repercussions. Phytomedicine 2004; 11**:**196-201.
12. Welt K, Weiss J, Martin R, Hermsdorf T, Drews S, Fitzl G. Ginkgo biloba extract protects rat kidney from diabetic and hypoxic damage. Phytomedicine 2007; 14:196-203.
13. Roger Walker, Clive Edwards. Clinical Pharmacy and Therapeutics, Edinburgh, Churchill Livingstone, 2001:635.
14. Eric T. Herfindal, Dick R. Gourley.Text Book of Therapeutics. Philadelphia, Lippincott Williams & Wilkins, 2001:82-383.