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# Hypoglycemic Effect of *Holarrhena antidysenterica* Seeds on Streptozotocin induced Diabetic Rats

**Supriya Mana[1]*, Sachin Singhal[1], Neeraj K.Sharma[1], Dharmendra Singh[1]**

[1]College of Pharmacy, Teerthanker Mahaveer University, Moradabad (U.P.)-244001, India

*Corres.author: manasupriya2007@gmail.com*

**ABSTRACT**: This study was undertaken to evaluate the hypoglycemic effect of methanolic extract of *Holarrhena antidysenterica* (MEHAD) seeds (Apocynaceae) in normal (Normoglycemic) and in streptozotocin (STZ) induced diabetic wistar rats. Oral administration of methanolic extract (250 mg/kg) for 18 days resulted in decrease in blood glucose level compared to the diabetic control. In addition, oral administration of the extract significantly decreased serum total cholesterol, triglyceride levels and at the same time markedly increased liver glycogen, thus proving the potent antidiabetic property of the plant.
**Keywords:** *Holarrhena antidysenterica* (HAD), Diabetes, Streptozotocin, Hypoglycemia.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism. It is the most common endocrine disorder and by the year 2010 it is estimated that more than 200 million people world wide will have DM and 300 million will subsequently have the disease by 2025[1]. Insulin and oral hypoglycemic agents like sulphonylureas and biguanides are still the major players in the management of the disease. However potent and specific agents from the pharmaceutical industry have not yet emerged. Alternative strategies to the current modern pharmacotherapy of DM are urgently needed[2]. Plants used in traditional medicine to treat DM represent a valuable alternative for the control of this disease.

*Holarrhena antidysenterica* (HAD) is a typical Indian medicinal plant. The bark and seeds are used to treat amoebic dysentery, diarrhea, asthma, bronchopneumonia, malaria[3]. Decoction of seeds was recommended by "Bhavaprakasha" in diabetes[4]. However no scientific data is available regarding the effect of HAD on the blood glucose levels? The present study was undertaken to explore the effect of methanolic extract of HAD seeds on blood glucose level and to determine the probable mechanism of action.

## 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. A voucher specimen of seeds has been deposited in museum of department of pharmacognosy, Acharya & B.M Reddy College of Pharmacy, Bangalore.

### Preliminary phytochemical screening[5,6]

Preliminary phytochemical screening revealed the presence of alkaloids, steroidal alkaloids, carbohydrates, sugar and oils.

### Preparation of methanolic extract of Holarrhena antidysenterica:

The methanolic extract was prepared by soxhlet extraction of 500 g seed powder in 1000 ml of methanol. 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 strains 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), humidity (45 ± 5° C) and 12 hr day: 12 hr night cycle as per CPCSEA guidelines[7]. Animals were allowed free access to food (standard pellet diet) and water *ad libitum*. The animals were divided into 4 groups of 10 rats each. The study was approved by Institutional Animal Ethical Committee of Acharya & B.M Reddy college of Pharmacy, Bangalore, India.

**Induction of Diabetes**

After 10 days acclimatization, male wistar rats (200-250 g) were kept for overnight fasting. Diabetes was induced in same animals (lightly anaesthetized with ether) by a single intravenous (i.v) injection of streptozotocin (Sigma Aldrich Chemicals Pvt. Ltd.) 35 mg/kg b.w dissolved in 0.1 (M) Citrate buffer (pH – 4.5). Diabetes was confirmed by estimation made after third day of STZ injection for serum glucose by a semiautoanalyzer (Metro Lab). Rats were considered as diabetic when their fasting glycemia was >250 mg/dl.

**Experimental design and treatment schedule**

The diabetic rats were divided randomly into 4 groups consisting of 10 animals each. The group without STZ treatment and treated with an equal volume of physiological saline and 2 % gum acacia during the experiment was taken as a solvent control (normal). The group of STZ induced rats treated with an equal volume of physiological saline and 2 % gum acacia was taken as negative control. While the group treated with glibenclamide (10 mg/kg) was taken as positive control. Another was group treated (p.o) with methanolic extract of *Holarrhena antidysenterica* (250 mg/kg) for 18 days.

**Statistical Analysis**

Results are expressed as the mean ± S.E. Differences between mean values were analyzed by Student's t-test or Dunnett's test and $P < 0.05$ was considered significant.

**RESULTS**

Changes in the body weight in different groups are shown in (table 1). Significant weight loss was observed in negative control group than normal control group. The groups of rats treated with methanolic extract of *Holarrhena antidysenterica* and Glibenclamide shown an increase in body weight compared to negative control group. Alteration in blood glucose level on treatment of diabetic rats with *Holarrhena antidysenterica* (seed methanolic extract) and Glibenclamide is given (table 2). The blood glucose was increased significantly in untreated STZ induced diabetic rats as compared to normal rats. Administration of methanolic extract of *Holarrhena antidysenterica* (seed) and Glibenclamide led to significantly decreased in blood glucose levels in diabetic treated group.

Significant difference was also observed in liver glycogen level and total protein level estimated in diabetic rats (table 3). A decrease in the serum triglyceride and cholesterol levels was observed (table 4).

**Table 1: Alteration in different body weight on treatment of diabetic rats with *Holarrhena antidysenterica* (seed methanolic extract) and Glibenclamide.**

| Group of Animals | Body Weight of Different Group (g) | | | |
|---|---|---|---|---|
| | 0 day | 6th Day | 12th Day | 18th 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 (10 mg/kg) | 193.17 ± 2.20* | 205.00 ± 2.29** | 209.68 ± 3.98** | 212.78 ± 2.08** |
| Diabetic + MEHAD (250 mg/kg) | 186.50 ± 3.53* | 193.00 ± 2.29** | 201.16 ± 6.13** | 217.17 ± 3.23** |

Values are the mean ± S.E.

* $P < 0.05$, * * $P < 0.01$ as compared to Normal control, Diabetic control.

**Table 2: Alteration in blood glucose level on treatment of diabetic rats with *Holarrhena antidysenterica* (seed methanolic extract) and Glibenclamide**

| Group of Animals | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|
| | Initial | 6th Day | 12th Day | 18th 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 (10 mg/kg) | 344.00 ± 11.78* | 327.16 ± 8.86* | 258.33±12.81** | 136.33 ± 3.26** |
| Diabetic + MEHAD (250 mg/kg) | 325.50 ± 2.07* | 311.33 ± 3.33* | 268.83 ±7.21** | 162.16 ± 3.30** |

Values are the mean ± S.E.
* $P < 0.05$, * * $P < 0.01$ as compared to Normal control, Diabetic control.

**Table 3: Alteration in liver glycogen level and total protein level on treatment of diabetic rats with *Holarrhena antidysenterica* (seed methanolic extract) and Glibenclamide.**

| Group of Animal | Live 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 (10 mg/kg) | 36.11 ± 0.98** | 5.91 ± 0.20** |
| Diabetic + MEHAD (250 mg/kg) | 44.07 ± 1.13** | 6.18 ± 0.13** |

Values are the mean ± S.E.
* $P < 0.05$, * * $P < 0.01$ as compared to Normal control, Diabetic control.

**Table 4: A decrease in the serum triglyceride and cholesterol levels on treatment of diabetic rats with *Holarrhena antidysenterica* (seed methanolic extract) and Glibenclamide.**

| 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 (10 mg/kg) | 141.77 ± 1.88** | 50.80 ± 0.22** |
| Diabetic + MEHAD (250 mg/kg) | 132.90 ± 7.22** | 55.80 ± 3.12** |

Values are the mean ± S.E.
* $P < 0.05$, * * $P < 0.01$ as compared to Normal control, Diabetic control.

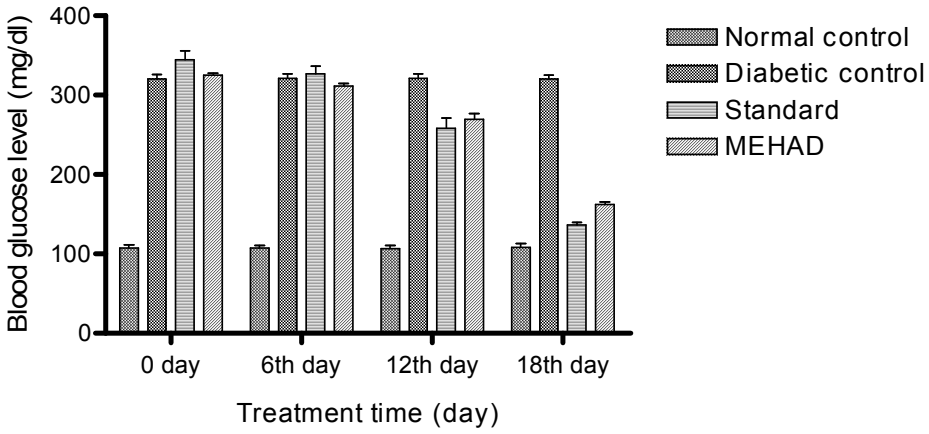


**Fig 1: Effect of blood glucose level on STZ induced diabetic rats.**

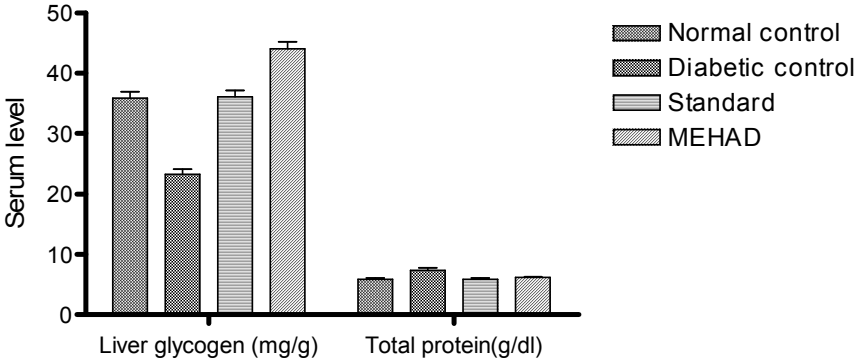


**Fig 2: Effect of extract on liver glycogen & total protein on STZ induced diabetic rats.**

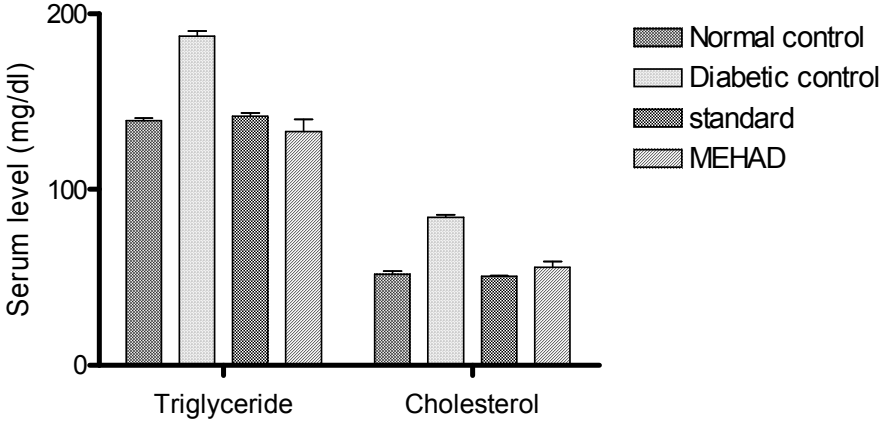


**Fig 3: Effect of extract on Triglyceride & cholesterol on STZ induced diabetic rats.**

## DISCUSSION

The diabetogenic action of STZ is mediated by selective destruction of pancreatic *beta* cells[8]. An observations in this study correlates with the previous finding, in that the blood glucose levels significantly increased in untreated STZ induced diabetic rats. In the present study, administration of methanolic extract of *Holarrhena antidysenterica* (seed) for a period of 18 days to diabetic rats showed a significant decreased in the levels of blood glucose. The possible mechanism by which methanolic extract brings about its hypoglycemic activity may be the potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing *beta* cells or by its release from the bound insulin[9]. We have noticed a significant increase in body weight in treated STZ diabetic rats. This could be the result of improved glycaemic control[10] proved by methanolic extract of *Holarrhena antidysenterica* (seed). It was observed that there was an increase in the triglyceride and cholesterol levels in diabetic rats. This might have occured 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].

In the absence of insulin, protein production is not favoured[14,15]. Significant reduction in liver glycogen was also observed in diabetic rats, which results in inactivation of glycogen synthestase system. Treatment with methanolic extract has shown a significant decreased in the serum cholesterol, serum triglycerides indicating an increase in insulin level. Also there was significant increase in total protein and liver glycogen levels. Thus representing the antidiabetic action of may be due to improvement of glycogenesis.

## CONCLUSION

Thus the present study indicates that treatment with methanolic extract of *Holarrhena antidysenterica* (seed) has favourable effect not only on blood glucose levels, liver glycogen but also on serum lipids and body weight. This point out the promising effect of *Holarrhena antidysenterica* seed being a useful antidiabetic agent and also in diabetic complications. Further studies are necessary to ascertain the use of *Holarrhena antidysenterica* seed in diabetic complications.

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