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Anti-diabetic activity of *Holarrhena antidysenterica* (Linn.) Wall, bark on alloxan induced diabetic rats

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Abstract

Objective: To screen the antidiabetic potential of the bark of *Holarrhena antidysenterica* Linn. Materials and methods: In the present study, bark of *Holarrhena antidysenterica* Linn was screened for antidiabetic activity. Bark powder of *H.antidysenterica* was subjected to hot continuous extraction (soxhlet) with various solvents like petroleum ether (40-60°C), chloroform, butanol, butanone and alcohol. Aqueous extract was prepared by cold maceration. After preliminary phytochemical investigation, all the extracts were evaluated for antidiabetic activity after single dose (acute study) and after prolonged treatment (chronic study) in alloxan induced diabetic albino rats. All the extracts were given orally at a dose of 250 mg/kg b.w., Glibenclamide was used as standard drug (10 mg/kg b.w. p.o.). Results and discussion: Alcohol, butanol, chloroform, aqueous and butanone showed significant antidiabetic activity in acute as well as prolonged treatment compared to control. Petroleum ether extract did not show significant antidiabetic activity on prolonged treatment. Among all the extracts, alcoholic extract had more significantly reduced the blood glucose level after single dose and nearly equal to standard Glibenclamide after prolonged treatment.

Key words: *Holarrhena antidysenterica,* Antidiabetic, Glibenclamide, Alloxan.

1. Introduction

Holarrhena antidysenterica (Linn.) Wall is a genus of trees or shrubs found in the tropics and subtropics of the old world [1]. It comprises seven or eight species, which are distributed in Asia, tropical areas of Africa, Madagascar, India, Philippines and Malayan Peninsula [2]. This small tree is common in the forest of India, indigenous to Himalayas, Assam, Uttar Pradesh, down to Travancore, Orissa and Maharashtra [3]. The plant is well known as 'Kurchi'. The bark is thick, brown and rough, with abundant milky white latex. The bark and seeds are bitter, constipating, astringent, acrid, refrigerant, anthelmintic, antiperiodic, aphrodisiac, carminative, expectorant, frebrifuge and tonic. They are useful in amoebic dysentery, diarrhoea, asthma, hepatopathy. The leaves are used in chronic bronchitis, boils, ulcers and dysentery [4].

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The survey of literature reveals that various parts such as roots, bark, leaves, flowers, fruits and seeds of *H.antidysenterica* (Linn.) Wall has been traditionally documented to possess antidiabetic activity [5]. Aqueous and alcoholic extracts of *H.antidysenterica* Wall seeds were scientifically proved to possess antidiabetic activity [6]. Fruit extract (50% ethanol) of *H.antidysenterica* was also showed hypoglycemic activity in rats [7].

But there is no scientific report for antidiabetic activity of bark of *H.antidysenterica.* Hence to ascertain the claim, bark of *H.antidysenterica* has been selected for evaluation of antidiabetic activity in the present study.

2. Materials and methods

2.1 Plant material

The bark of *H.antidysenterica* was collected from the local areas of Belgaum, identified and authenticated from botanist Prof. R.S. Goudar, Dept. of Botany, R.L. Science Institute, Belgaum. A specimen voucher (No. SB 01) of the same is deposited in the Dept. of Pharmacognosy, K.L.E.S's College of Pharmacy, Belgaum.

2.2 Extraction of plant material

The bark of *H.antidysenterica* was shade dried and powdered to desired size. Powdered air-dried bark of plant subjected to hot continuous batch extraction (soxhlet extractor) with various solvents like petroleum ether (40-60°C), chloroform, butanol, butanone and alcohol. Aqueous extract was prepared by cold maceration. After complete extraction, the solvents were distilled off and concentrated to obtain residue. For each time of extraction, fresh powdered bark of plant was used.

2.3 Antidiabetics screening

2.3.1. Acute toxicity study

The acute oral toxicity study was carried out as per OECD guidelines. 250 mg/kg b.w. of each extract was taken as effective dose for evaluation of antidiabetic activity [8].

2.3.2. Preparation of dose

The petroleum ether (40-60°C), chloroform, butanol, butanone, alcohol and aqueous extracts (250 mg/kg b.w.) were formulated as suspension in distilled water using Tween-80 as a suspending agent since Tween-80 has negligible effect on normal blood glucose level (BGL). The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

2.3.3. Preparation of standard drug

Glibenclamide was used as the reference drug for evaluating the antidiabetic activity. Daonil tablets (10 mg) formulated by Aventhis Pharma Ltd., Goa, were powdered and made into suspension in distilled water using Tween-80 as a suspending agent. The strength of suspension was prepared according to 10 mg/kg b.w.

2.3.4. Animals

Wistar albino rats of either sex weighing 150-200 g were selected for screening antidiabetic activity. Animals were fed standard diet and water *ad libitum* during entire period of experimentation. Alloxan monohydrate (100 mg/b.w.) was used orally to induce hyperglycemia.

2.3.5 Methodology [9-10]

Before starting the experiment, animals were separated according to their body weight. The animals were segregated into nine groups of six rats each, taking into consideration of diabetic blood sugar level. One group was normal control and others were diabetic control along with petroleum ether, chloroform, butanol, butanone, alcohol and aqueous extracts, which were compared with standard Glibenclamide group. The acclimatized animals were kept fasting for

24 h with water *ad libitum* and injected intraperitoneally at dose of 100 mg/kg b.w. of alloxan monohydrate freshly prepared in normal saline solution.

After one hour of alloxan administration, animals were given fed *ad libitum* and 1ml of (100 mg/ml) glucose i.p. to combat ensuring severe hyperglycemia. After 72 h of the alloxan injection, the animals were tested for evidence of diabetes by estimating their blood glucose level by using glucose estimation kit. The blood glucose level more than 150 mg/100 ml of blood was criteria.

Test extracts (250 mg/kg b.w.), standard drug Glibenclamide (10 mg/kg b.w.) and vehicle were administered orally, every 24 h for period of seven days. The blood samples were obtained through the tail vein puncturing with hypodermic needle. 0.2 ml of blood was withdrawn at interval of initial 1, 3, 5 and 7th h of administration of single dose (for acute study) and at the end of 7th day (prolonged treatment).

The blood glucose level was measured in all the groups by using "Glucose enzyme reagent system", manufactured by Span Diagnostic Private Ltd., Surat, India. The system uses glucose oxidase method of estimating glucose in blood.

2.3.6 Statistical analysis

Data obtained was subjected to One Way ANOVA followed by Dunnet's 't' test to determine the statistical significance of the change in blood glucose level.

Table No.1
Effect of *H. antidysenterica* bark on blood glucose level of alloxan induced diabetic albino rats after single dose.

Group (n)	Dose	Blood Glucose Level mg/100ml (Mean ± SEM)				
		Initial	1 h	3 h	5 h	7 h
Normal control	2 ml saline	98.56± 0.874	99.32± 0.866	99.4± 0.950	99.91± 1.288	99.74± 1.133
Diabetic control	2 ml saline	203.6± 3.850	208.3± 4.483	213.0± 3.83	217.8± 4.03	222.0± 4.058
Petroleum ether extract (40 - 60°C)	250 mg/kg b.w.	215.0± 4.022	213.8± 4.316	211.2± 4.227	208.6± 4.275	206.7± 4.427*
Chloroform extract	250 mg/kg b.w.	204.6± 4.162	203.0± 4.239	200.0± 4.167	196.1± 3.953**	191.7± 4.009**
Butanol extract	250 mg/kg b.w.	203.3± 3.697	202.4± 3.382	199.2± 3.401	193.2± 3.158**	184.8± 3.818**
Butanone extract	250 mg/kg b.w.	217.0± 3.592	217.3± 3.898	210.9± 3.22	206.0± 3.128	200.0± 2.686**
Alcohol extract	250 mg/kg b.w.	202.0± 3.796	200.3± 3.84	192.5± 4.311**	183.2± 4.011**	178.3± 2.916**
Water extract	250 mg/kg b.w.	205.7± 3.042	203.5± 3.201	200.0± 3.282	198.5± 2.571**	195.0± 2.897**
Glibenclamide	10 mg/kg b.w.	197.3± 3.924	193.8± 4.35*	187.8± 3.906**	180.5± 3.617**	171.8± 2.583**

*p<0.05 - Significant, **p<0.01 – More significant Vs. Diabetic Control; SEM: Standard Error Mean, n = Number of animals in each group (6)

Table 2.
Effect of *H. antidysenterica* bark on blood glucose level of alloxan induced diabetic albino rats after prolonged treatment.

Group (n)	Dose	Blood Glucose Level mg/100ml (Mean±SEM)	
		Initial	7th day
Normal control	2 ml saline	98.56 ± 0.874	86.97 ± 2.971
Diabetic control	2 ml saline	203.6 ± 3.850	210.7 ± 3.243
Petroleum ether extract	250 mg/kg b.w.	215.0 ± 4.022	205.5 ± 1.286
Chloroform extract	250 mg/kg b.w.	204.6 ± 4.162	172.9 ± 5.058**
Butanol extract	250 mg/kg b.w.	203.3 ± 3.697	166.9 ± 3.163**
Butanone extract	250 mg/kg b.w.	217.0 ± 3.592	188.9 ± 1.819**
Alcohol extract	250 mg/kg b.w.	202.0 ± 3.796	162.7 ± 1.471**
Aqueous extract	250 mg/kg b.w.	205.7 ± 3.042	180.9 ± 2.280**
Glibenclamide	10 mg/kg b.w.	197.3 ± 3.924	160.8 ± 2.553**

*p<0.01 – Significant, **p<0.001 – More significant Vs. Diabetic Control SEM: Standard Error Mean, n = Number of animals used in each group (6)

3. Results and Discussion

The results are expressed as change in blood glucose level presented in table no.1 and 2. The results obtained from alloxan-induced diabetes indicated that alcohol, butanol, chloroform, water and butanone showed more significant ($p<0.01$) antidiabetic activity in acute as well as prolonged treatment compared to diabetic control. The results were comparable with reference standard Glibenclamide. Petroleum ether extract did not show significant activity on prolonged treatment but showed significant ($p<0.05$) activity at 7th h in acute study compared to diabetic control.

The single dose of alcoholic extract (250 mg/kg b.w.) has more significantly reduced the blood glucose level at 3rd h (202±3.796 at 0 h to 192.5±4.31 at 3rd h) and significant hypo-glycemia was maintained for another 4 h. Glibenclamide (10 mg/kg b.w.) has also significantly reduced blood glucose level at 3rd h (197.3±3.924 at 0 h to 187.8±3.906) and significant hypoglycemia maintained for another 4 h.

On prolonged treatment, the effect of alcoholic extract (162.7±1.471) was nearly equal to that of reference drug Glibenclamide (160.8± 2.553). These findings clearly established that alcoholic extract exhibited more potent antidiabetic activity than all other extracts as it contains constituents such as alkaloids, steroids and tannins. As per literature alkaloids, sterols and tannins are known to reduce blood glucose level in diabetic condition [11,12]. Hence the more potent antidiabetic activity of *H.antidysenterica* bark may be due to nature of alkaloids, sterols or tannins present in the bark.

However, this claim demands further research to isolate antidiabetic principle, since the present study was preliminary investigation.

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