Anti-diabetic activity of ethanolic extract of Holostemma ada Kodien Schults in alloxan induced diabetic rats

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Abstract

Object: To evaluate antidiabetic activity of ethanolic extract of Holostemma ada Kodien Schults (EEHK) in normal, glucose fed , alloxan-induced diabetic rats and to perform phytochemical and toxicity studies. Material and Methods: The alcoholic extract ofHolostemma ada Kodien (Asclepiadaceae) was studied for antidiabetic activity in normal, glucose fed and alloxan- induced diabetic rats by oral administration of extract (200 and 400 mg/kg body wt) for 7 days. The effect was compared with 0.5 mg/kg (i.p) glibenclamide.Results: The alcoholic extract ofHolostemma ada Kodien significantly lowered the blood sugar of hyperglycemic rats. From toxicity study it was observed that EEHK was non toxic upto 5 g/kg body weight and phytochemical studies shows the presence of alkaloids, flavonoids, flavanones, tannins, terpenoids, amino acids and carbohydrates.Conclusion: The results justified the traditional use in the treatment of diabetes.

INTRODUCTION

Diabetes mellitus (DM) is considered to be one of the most serious endocrine syndrome. In many countries it is traditional to use plants to control diabetes [1234] . The anti hyperglycemic effect of several plant extracts which were used as antidiabetic remedies has been confirmed [[[5,6,7,8.]]] . The synthetic hypoglycemic agents used in clinical practices have serious side effects like hematological effects, coma, disturbs the functions of liver and kidney. In addition they are not suitable for use during pregnancy [9] . Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects [10] . Therefore, the search for more effective and safer antidiabetic agent has become an area of active research.

Holostemma ada Kodien, important medicinal plant belonging to family Asclepiadaceae and widely distributed in tropical forest in India [1112] . The plant is used as antidiabetic [10] , rejuvenative, aphrodisiac, expectorant, galactogogue, stimulant, and in ophthalmic disorders [14] . There is huge demand for this plant; more than 150 tonnes is required every year in south Indian pharmacies [15] . However no scientific study on anti diabetic activity of this plant has been reported. The present investigation was undertaken to study the anti diabetic activity of Holostemma ada Kodien in alloxan induced diabetic rats.

MATERIALS AND METHODS

PLANT MATERIAL

Fresh leaves were collected from S.V.U campus, Tirumala gardens of Chittor district of Andhra Pradesh of India and authentified by Asst.Prof.Dr.K.Madhava Chetty of the Department of Botany, S.V.University, and Tirupathi. A.P. A voucher specimen [No.HAK1/PRRMCP 06-11] was deposited at Department of Pharmacognosy for further reference.

EXTRACTION

The leaves, shade dried, powdered in a grinder mixer to obtain a coarse powder and then passed through 40 mesh sieve. The powdered leaves (430gms) were defatted with hexane and later extracted (soxhlet) using alcohol. The extract evaporated to dryness, gave a residue 15.5%w/w. Phytochemical screening [16] were performed.

ANIMALS

Albino wistar rats of either sex weighing (200-250gms) were employed for study. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. Ethical clearance for animal study was obtained from institutional animal ethical committee. (IAEC/PRRMCP/2006/07).

TOXICITY STUDY

An acute toxicity study was performed to determine the LD_{50} using different doses of the extract according to the method described by Ghosh et.al [17] .

EFFECTS OF EEHK ON BLOOD GLUCOSE LEVELS IN NORMOGLYCEMIC RATS

Animals were divided into three groups of six rats in each group. Group-1: Animals received 1% SCMC 2 ml/kg body wt. per orally. Group-2: Animals received EEHK 200 mg/kg body wt. per orally.Group-3: Animals received EEHK 400 mg/kg body wt. per orally.

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the above mentioned dosage schedule. Blood glucose levels were determined at 0 (before drug challenge) 60, 120 min, after drug administration

EFFECT OF EEHK ON BLOOD GLUCOSE LEVEL ON GLUCOSE FED HYPERGLYCEMIC RATS (ORAL GLUCOSE TOLERANCE TEST)

The animals were divided into four groups of six rats in each group. Group-1: Animals received glucose at a dose 2 gm/kg body wt. per orally. Group-2: Animals received glibenclamide 0.5 mg/kg body wt. and glucose Solution at a dose 2 gm/kg body wt. per orally. Group-3: Animals received EEHK 200 mg/kg body wt. and glucose Solution at a dose 2 gm/kg body wt. per orally. Group-4: Animals received EEHK 400 mg/kg body wt. and glucose Solution at a dose 2 gm/kg body wt. per orally. In this study, the entire group of animals were fasted and treated with above dosage schedule orally. The EEHK 200 mg/kg, 400 mg/kg and 0.5 mg/kg glibenclamide were administered half an hour before administration of glucose solution. Blood glucose levels were determined at 0(before glucose challenge) 30, 60, 90, 120 th mins after glucose administration.

EFFECT OF EEHK ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RATS

Different groups of rats were used to study the effects of EEHK. The rats were divided into five groups each consisting of six rats. Group-1: Normal control animals received 1% SCMC 2 ml/kg body wt. per orally. Group-2: Alloxan (150 mg/kg body wt.) induced diabetic animals received 1% SCMC 2 ml/kg body wt. per orally. Group-3: Alloxan (150 mg/kg body wt.) induced diabetic animals received glibenclamide 0.5 mg/kg, body wt. per orally. Group-4: Alloxan (150 mg/kg body wt.) induced diabetic animals received EEHK 200 mg/kg body wt. per orally. Group-5: Alloxan (150 mg/kg body wt.) induced diabetic animals received EEHK 400 mg/kg body wt. per orally.

In this study all the surviving diabetic animals and normal animals were fasted over night. Blood samples were collected from the fasted animals prior to the treatment with above schedule and after administration at each day up to 7days. For glucose determination, blood was obtained by snipping tail with sharp razor [18] using Haemo-Glukotest (20-800R) glucose strips supplied by M/s Boehringer Mannheim India Ltd. This method, which permits the measurement of blood glucose levels with minimum injury to rat, was previously validated by comparison with glucose oxidase method [1920] .

STATISTICAL ANALYSIS

All values were expressed as mean ± SEM .The data were statistically analysed by ANOVA followed by Dunne's -'t' test [21] .

RESULTS

PHYTOCHEMICAL AND TOXICITY STUDIES

Phytochemical screening gave positive results for alkaloids, flavonoids, flavanones, tannins, terpenoids, amino acids and carbohydrates. In toxicology study it was observed that extract is non toxic upto 5 g/kg body weight.

EFFECTS OF EEHK ON BLOOD GLUCOSE LEVELS IN NORMOGLYCEMIC RATS

At dose 200 mg/kg and 400 mg/kg of EEHK in fasting rats, blood sugars level were assessed in normal rats at various time intervals. The results were shown in Table-1.The mean blood glucose level maintained at 83.00 mg/dl at dose of 200 mg/kg body weight of EEHK and decreased from 88.60 mg/dl to 84.00 mg/dl at dose of 400 mg/kg body weight in rats treated with EEHK.

Figure 1

Table – 1 Effect of EETC on Blood glucose in normoglycemic rats

GROUPS	Blood glucose levels (mg/dl)		
	Initial	60min	120 min
I	86.00 ±0.57	87.50 ±0.42	85.80±0.49
II	83.00 ±0.57	84.80 ±0.47	83.00 ±0.36
III	88.60 ±0.88	82.85±0.99	84.00 ±0.96

The values are expressed as mean ± SEM. n = 6 number of animals in each group.

Effect of EEHK on blood glucose level on glucose fed

hyperglycemic rats (oral glucose tolerance test)

At dose 200 mg/kg and 400 mg/kg of EEHK blood sugars level were assessed in glucose fed rats at various time intervals. The results were shown in Table-2.The mean blood glucose level decrease from 88.33 mg/dl to 86.17 mg/dl at dose of 200 mg/kg body weight of EEHK and 90.67 mg/dl to 85.44 mg/dl at dose of 400 mg/kg body weight in rats treated with EEHK, which is comparable to standard drug administration which shows reduction of mean blood glucose level from 86.16 mg/dl to 80.67 mg/dl.

Figure 2

Table – 2 Effect of EETC on Blood glucose in glucose fed hyperglycemic normal rats (oral glucose tolerance test):

Groups	Blood glucose levels (mg/dl)				
	Initial	30 min	60min	90 min	120 min
I	84.33±0.60	120.60±1.47	124.30±1.22	110.16±2.01	88.17±1.92
II	86.16±0.98	120.20±1.15	104.50±1.90*	92.33±0.70*	80.67±0.89*
III	88.33±2.02	126.5±1.41	108.30±1.19*	97.33±0.89*	86.17±1.03*
IV	90.67±0.89	124.5±1.09	106.4±1.02*	94.13±1.05*	85.44±1.67*

The values are expressed as mean ± SEM .n =6 number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's –'t' test. The blood glucose values of group II, III and IV are compared with control animal's values *P<0.05 were taken as Significant.

EFFECT OF EEHK ON BLOOD GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RATS

The antihyperglycemic effect of the extracts on the blood sugar level on diabetic rats is shown in Table-3. The blood glucose level of diabetic animals significantly reduced from 207.30 mg/dl to 105.10 mg/dl at 200 mg/kg body wt. of EEHK and 212.83 mg/dl to 89.16 mg/dl at 400mg/kg body wt. of EEHK. These results were comparable with 0.5mg/kg of glibenclamide which shows significant reduction from 213.00 mg/dl to 86.16 mg/dl on 7 th day.

Figure 3

Table – 3 Effect of EETC on Blood Glucose level in Diabetic Rats

Groups	Blood glucose levels (mg/dl)						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
I	85.00 ± 0.88	86.66 ± 0.88	86.16 ± 0.30	85.50 ± 0.56	85.16 ± 0.60	86.00 ± 0.57	85.60 ± 0.49
II	216.60 ± 2.25	229.16 ± 3.01	232.50 ± 2.81	263.30 ± 3.25*	277.50 ± 2.14**	296.66 ± 2.47**	315.80 ± 2.81**
III	213.00 ± 2.15	198.80 ± 1.332	180.16 ± 2.20	153.00 ± 2.76#	131.16 ± 2.14**	106.00 ± 1.39**	86.16 ± 0.22**
IV	207.30 ± 1.70	194.60 ± 1.95	188.52 ± 1.50	163.60± 1.61	135.16 ± 1.62*	121.00 ± 2.12**	105.10 ± 0.25**
V	212.83 ± 2.20.	193.83± 2.65	176.30 ± 2.07	150.50 ± 2.04	130.50 ± 1.89#	108.33± 1.20**	89.16 ± 0.90**

The values are expressed as mean ± SEM. n = 6 number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's –'t test. The blood glucose values of group III, IV and V are compared with control animal's values**P<0.001, *P<0.01, #P<0.05.

DISCUSSION

In the recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential by various investigators in experimental animals. These properties were attributed to different formulations, extracts and active principles. Working on the same line, we have undertaken a study on Holostemma ada Kodien for its anti-diabetic property.

Preliminary phytochemical analysis of the ethanolic extract of the Holostemma ada Kodien showed that the plant has a rich possession of phytochemicals like alkaloids, flavonoids, flavanones, tannins, terpenoids, amino acids and carbohydrates. Acute oral toxicity studies revealed the non-toxic nature of the ethanolic extract of Holostemma ada Kodien. Neither lethality nor any profound toxic reactions was observed at a dose of 5000 mg/kg body wt. This indirectly pronounces the safety profile on the plant extract.

The ethanolic extract at a dose of 200 mg/kg body wt. per orally did not significantly suppress blood glucose levels in over night fasted normoglycemic animals. The same effect was observed at a higher dose of 400 mg/kg body wt. per orally of the EEHK in over night fasted normoglycemic animals after 1 st and 2 nd hour of oral administration, when compared with control group of animals.

The ethanol extract showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and

glycogenic process with concomitant decrease in glycogenolysis and glyconeogenesis [22] . However the effect was less significant when compared to standard drug glibenclamide.

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan causes diabetes by rapid depletion of cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release, thereby a drastic reduction in plasma insulin concentration leading to stable hyperglycemic state [23] . In this study significant hyperglycemia was achieved within 48 hours after Alloxan (150 mg/kg body wt. i.p.) injection. Alloxan induced diabetic rats with more than 200 mg/dl of blood glucose were considered to be diabetic and used for the study.

The studies on antidiabetic activity in alloxanised rats showed significant reduction of blood glucose level from the 4 [th] day of the study. The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extra pancreatic effects [24] . From the phytochemical analysis it was found that the major chemical constituents of the extract were flavonoids and tannins. Over 150 plant extracts some of the active principle including flavonoids are known to be used for the treatment of diabetes [25262728] . On the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed anti diabetic activity [2930] .

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References

1. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti diabetic potential. J Ethanopharmacol 2002; 81:81.
2. Grover JK, Yadav SP. Pharmacological actions and potential uses of momordi charantia : a review. J Ethanopharmacol 2004; 93:123.
3. Jia W, Gao WY, Yan YQ, Wang J, Xu ZH, Zheng WJ, et al. The rediscovery of ancient Chinese herbal formulas. Phytother Res 2004; 18:681.
4. Platel K, Srinivasan K. Plant foods in the management of diabetes mellitus: vegetables as potential hypoglycemic agents. Nahrung 1997; 41:68.
5. Virdi J, Sivakami S, Shahani S, et al. Anti hyperglycemic effects of three extracts from momordi charantia. J Ethanopharmacol 2003; 88:107.
6. Pari L, Ramakrishna R, Venkateswaran S. Anti hyperglycemic effects of diamed, a herbal formulation in experimental diabetes in rats J Pharm Pharmcol 2001; 53:1139.
7. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hyperglycemic activity of Indian medicinal plants in alloxan diabetic rats. J Ethanopharmacol 2003; 84:1435.
8. Babu PS, Stanely Mainzen Prince P. Anti hyperglycemic and anti oxidant effects of hyponiad, an ayurvedic herbomineral formulation in streptozocin induced diabeteic rats. J Pharm Pharmcol 2004; 56:1435.
9. Larmer J. insulin and oral hypoglycemic drugs, glucogan .In: Gilman AG, Goodman LS, Rall TW, Murad F, Editors. The pharmacological basis of therapeutics .7th Edition Newyork: Macmillan Publishing; 1985, 1490.
10. Moming A. role of indigenous medicine in primary health care. Proceeding of first international seminar on unani medicine; 1987. New Delhi, 54.
11. Kolammal M. Pharmacognosy of Ayurvedic drugs. Dept. of Pharmacognosy, University of Kerala, Trivandrum 1979:21.
12. Sivarajan VV, Balachandran I. Ayurvedic drugs and their plant sources. Oxford and IBM Pub.co. Pvt. Ltd. New Delhi 1994:195
13. Madhava CK, Sivaji K, Tulasi rao. Flowering plants of Chittor district – Andhra Pradesh, India, 1st ed. Students offset printers, Tirupathi 2008:33.
14. Warrier PK, Nambiar VPK, Raman KC. Indian medicinal plants: A compendium of 500 species. Orient Longman 1995; 3:165.
15. Karmarkar SH, Keshavachandran R, Augustin A. Biochemical evaluation of root tubers and in vitro induced callus of Adapathiyan (Holostemma ada Kodien K. Schum). J of Tropical Agriculture 2001; 39; 108.
16. Trease EG, Evans WC. Pharmacognosy, 13th edition London:Bailliere Tindall, 1989.386.
17. Ghosh MN. Fundamental of experimental Pharmacology 2nd ed. Scientific book agency: Calcutta: India 1984:53.
18. Aydin E, Fahrettin K, Hulusi A, Husseyin U, Yalcin T, Muzaffer U. Hypoglycaemic effect of Zizyphus jujube Leaves. J Pharm Pharmacol 1995:4772.
19. Jayakar B, Suresh B. Antihyperglycemic and hypoglycemic effect of Aporosa indleyana in normal and alloxan induced diabetic rats. Journal of Ethanopharmacology, 2003: 84:247.
20. Porchezhian E, Ansari SH, Shreedharan NKK, Antihyperglycemic activity of Euphrasia officinale leaves. Fitoterapia 2000; 71:522.
21. Saunders WB, Trapp GR.Basic and clinical biostatistics, 2nd ed. London Prentice Hall International 1993:99.
22. Luzi L, Pozza G. Glibenclamide: An old drug with novel mechanism of action. Acta Diabetologica 1997; 34:239.
23. Szkudelski T. The mechanism of alloxan and streptozotocin action in B-cells of the Rat pancreas. Physiol Res 2001; 50:536.

24. Senapati AK, Dash GK, Ghosh T, Christina AJM. A study on anti inflammatory and
Hypoglycemic activity of Bougainvillea spctabilis. Indian J. Nat. Prod. 2006; 22:3.
25. Meiselman HL, Halperrn BP, Dateo GP. Reduction of sweetness judgement by
extracts from the leaves of Ziziphus jujube Physiology and Behavior 1976;17:313.
26. Choi JS, Yokozawa T, Oura H. Improvement of hyperglycemia and hyperlipidmia in streptozocin – diabetic rats by methanolic extract of Prunus
davidiane stems and its main component, pruning. Planta Med 1991; 57; 208.
27. Ernmenisogiu A, Kelestimur F, Koker AH, et al. Hypoglycemic effect of Ziziphus
Jujube leaves. J Pharm pharmacology 1995; 47: 72-74.
28. Suba V, Murugasen R, Bhaskara Rao. et al. Antidiabetic potential of Barleria
lupuline in rats. Fitoterapia 2004; 75: 1.
29. An Iwu MM.Hypoglycemic property of Beridelia furruginear leaves. Fitoterapia
1983; 54:243.
30. Iwu MM.Antidiabetic properties of Beridelia furruginear leaves. Plant Med 1980;
39: 24.

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