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Evaluation of Anti-Diabetic Activity of the Plant Leaves of Verbascum thapsus in Alloxan Induced Diabetic Rats

Anusha Pothamsetty[1], M. Janarthan[1], Md. Faheemuddin[1*], Mohsina Hussain[2], Md. Mazher Ahmed[3]

[1]Department of Pharmacology, Nimra College of Pharmacy, Jawaharlal Nehru Technical University Kakinada, Kakinada-533003, Andhra Pradesh, India
[2]Department of Pharmacy Practice, Smt. Sarojini Ramulamma college of pharmacy, Palamur University, Mahbubnagar-509001, Telangana, India
[3]Department of Pharmaceutics, Luqman College of Pharmacy, Gulbarga 585101, Karnataka, India

[*]**Corresponding author:** Faheemuddin M, Department of Pharmacology, Nimra College of Pharmacy, Jawaharlal Nehru Technical University Kakinada, Kakinada-533003, Andhra Pradesh, India, E-mail: faheemuddin.md4u@gmail.com

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Abstract

Objective: *To evaluate the anti-diabetic activity of ethanolic extract of Verbascum thapsus (L) on alloxan induced diabetic rats.*

Methods: *Diabetes was induced in Wistar rats by intraperitoneal injection of alloxan monohydrate (100 mg/kg b.w/i.p). Ethanolic extract of Verbascum thapsus (L) (250, 500 mg/kg b.w/p.o) was prepared freshly, administered to alloxan induced diabetic rats for 21 days. Blood glucose levels monitored at 1, 3, 7, 14 and 21 days, serum lipid profile and Histopathological changes in pancreas were examined after 21 days. OGTT was performed by administration of 250 and 500 mg/kg b.w/p.o of ethanolic extract of Verbascum thapsus (L) and 10 mg/kg b.w/p.o of Glibenclamide to different groups respectively in normal rats.*

Results: *Significant ($p<0.001$) results were observed in the estimated parameters like reduction in blood glucose, elevated cholesterol, triglyceride, VLDL, LDL levels and also increase in the levels of HDL were observed in diabetic rat's treatment after 21 days of extract. The treatment produced protective effect of β-cells of Langerhans of pancreas in rats by histopathological studies. Oral glucose tolerance test, blood glucose levels significantly lower at all time points (In extract and standard Wistar rats) that blood was sampled after oral glucose load.*

Conclusion: *The results were suggested that the whole plant extract of Verbascum thapsus (L) having potent Antidiabetic activity on alloxan-induced diabetic rats and this justifies its use in ethanomedicine and can be exploited in the management of diabetes.*

Keywords: *Verbascum thapsus, Alloxan, Diabetic rats, Glibenclamide*

Introduction

Diabetes mellitus is a group of metabolic disorders in which a person has high blood sugar, either body does not produce enough insulin, or cells do not respond to the insulin that is produced [1-3]. Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double [1-3].

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. About 75-80% of the world population, mainly in the developing countries still use plant based medicines for primary health care [4]. Based on a large number of chemical and pharmacological research work, numerous bioactive compounds have been found in medicinal plants for diabetes. A number of investigators have shown that cumarins, flavonoids, terpenoids, and a host of other secondary plant metabolites, including arginine and glutamic acid, possess hypoglycemic effect in various experimental models [5,6]. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive [7].

Materials and Methods

The plant *Verbascum thapsus* Fresh leaves are collected from Sri Venkateshwara University Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library.

Preparation of plant extract

Fresh plants leaves were collected, washed to remove adhered dirt, rinsed with distilled water, blotted and dried in shade. The shade-dried specimens were powdered in a mixer. This powder was subjected to Soxhlet extraction using 70% ethanol as solvent. This cycle was repeated many times, over hours or a few days. The extracts were concentrated under reduced pressure and preserved in refrigerator until further use. At the end of the hot extraction process each extract was filtered. The extracts were then kept in desiccators to remove remaining moisture, if present, and finally stored in air tight containers at 4^0C for further use [8].

Phytochemical screening

Phytochemical screening of crude extract was carried out employing standard procedures to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, saponins, Tannins, glycosides, carbohydrates and others [9].

Animals

Healthy adult male Wistar rats of 150-180 gm were selected for the study. The animals were housed in standard cages and kept under standard condition. They were given a standard diet and water *ad libitum*. Animal studies had approval of IAEC, Nimra College of Pharmacy constituted by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [8,10,11].

Determination of acute oral toxicity studies

The LD (50) of the extract was determined by using wistar rats. Rats were kept for overnight fasting prior to drug administration. A total of five animals were used, which received a single oral dose (2000 mg/kg/b.w) of *Verbasum thapsus* extract. After the administration of extract food was withheld for further 3-4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily therefore for a period of 14 days [8.12].

Hypoglycemic activity in normal rats (OGTT) and Alloxan induced diabetic rats: Oral Glucose Tolerance Test (OGTT)

In five groups (each group of N=6) overnight fasted normal animals the Oral glucose tolerance test (OGTT) was performed. 1st group was administered with distill water, 2nd group was given glucose 2 mg/kg b.w and the following group were treated with following treatment to one hour previously administration of glucose 3rd group was treated with glibenclamide (10 mg/kg bw) 4th and 5th group

was administered with 250 mg/kg b.w 500 mg/kg b.w ethanolic extract of *Verbascum thapsus*. **Table 1** showed the blood glucose levels of the Normal control (Normal saline 0.5 ml/kg b.w/p.o), Glibenclamide (10 mg/kg b.w/p.o) and ethanolic extract of *Verbascum thapsus (25*0 mg and 500 mg/kg b.w/p.o) at different time points (0, 30, 60, 120, 180 min) after oral administration of glucose (2 g/kg b.w/p.o). Blood was withdrawn from the tail vein glucose levels were estimated using Gluco check blood glucose monitoring kit. There was a peak increase in the blood glucose at 30 min in all the groups [13].

Table 1: Blood glucose levels of the Normal control, Glibenclamide and ethanolic extract of *Verbascum thapsus*

Group	Blood glucose level (mg/dL)				
	0 Min	**30 Min**	**60 Min**	**120 Min**	**180 Min**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
I-Normal normal Saline (0.5 ml/kg b.w/p.o)	82.8 ± 1.35	85.0 ± 1.95***	82.0 ± 1.91***	80.0 ± 1.64***	79.0 ± 1.11***
II-Control (oral glucose of 10 mg/kgb.w/p.o)	84.6 ± 1.645	121.0 ± 1.2***	145.0 ± 1.54***	140.0 ± 1.75***	138.0 ± 1.45***
III-Standard Glibenclamide (10 mg/kg b.w/p.o)	83.8 ± 1.35	125.0 ± 1.2***	134.0 ± 1.54***	112.0 ± 1.65***	82.0 ± 0.856
IV-Extract Treated (250 mg/kg b.w/p.o)	83.0 ± 1.83	122.0 ± 1.62***	135.0 ± 0.989***	107.0 ± 1.48***	96.5±0.764
V-Extract Treated (500 mg/kg b.w/p.o)	80.7 ± 1.31	105.0 ± 2.16***	123.0 ± 1.67***	97.7 ± 0.882***	81.7 ± 1.12**

Values are Mean ± SEM; n=6; *P value <0.01; **p <0.05; ***p < 0.001 *vs.* 0 min; mg/kg b.w/p.o: milligram per kilogram of body weight per oral.

Induction of diabetes and treatment

Healthy Wistar strain albino rats were selected and randomly divided into different groups with six animals in each group serving as group 'A'=normal, Control group='B', Standard= group 'C', Group 'D'=Ethanolic extract, 250 mg/kg b.wt; group 'E'=Ethanolic extract, 500 mg/kg b.wt. Alloxan monohydrate was first weighed individually for each animal according to its weight and then solubilised with 0.2 ml saline just prior to injection (**Table 2**). Diabetes was induced by injecting it at a dose of 100 mg/kg b.w/i.p. After 1 h of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation, and after 48 h, blood glucose was measured. One group served as a control which received vehicle alone. The diabetic rats (glucose level > 150 mg/dL) were separated and divided into different groups for experimental study [14].

Measurement of serum lipid profile

The serum from the blood was separated as under: Sample was collected (preferably in eppendorf tubes) The serum was centrifuged at 1000 rpm for 5 min. The serum was pipette out using a micropipette. The serum was labeled with the animal number and the estimations were made. The serum glucose level and the lipid profile (total **c**holesterol HDL, LDL, VLDL and triglyceride level) was determined enzymatically on prietest bio chemistry analyser [15,16].

Histopathological studies

At the end of the study i.e. on 28th day the rats were sacrificed and the tissues (pancreas) were collected. The whole histopathological process was carried out in accordance with the SOPs (Standard Operating Procedures) (Tissue fixation, Processing and Embedding) [17,18].

Table 2: Effect of treatment on Alloxan induced diabetic rat

Group	Blood glucose level (mg/dL)					
	0 day	**1st day**	**3rd day**	**7th day**	**14th day**	**21st day**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
I (Normal control)	88.00 ± 2.408	86.83 ± 2.344	86.50 ± 2.349	87.00 ± 2.366	87.17 ± 2.088	86.67 ± 2.319
II (Diabetic control)	241.8 ± 2.638	246.3 ± 2.431	256.8 ± 2.638	270.3 ± 2.404	283.0 ± 2.817	313.8 ± 4.301
III (Standard)	217.80 ± 2.561	194.30 ± 2.692***	170.20 ± 2.822***	142.30 ± 2.813***	119.80 ± 1.815***	86.67 ± 4.104***
IV (Test-I)	230.0 ± 4.367	217.6 ± 4.151	204.7 ± 4.631**	183.7 ± 4.773***	163.3 ± 4.566***	134.8 ± 4.377***
V (Test II)	232.20 ± 3.270	218.2 ± 3.198*	192.2 ± 3.420***	159.3 ± 5.162***	122.7 ± 2.616***	89.67 ± 3.844***
Values are Mean ± SEM; N=6; *P value< 0.01; **p <0.02; ***p < 0.001 *vs.* Diabetic control.						

Statistical analysis

The values obtained from the biochemical analysis were expressed as mean ± standard Error of Mean (S.E.M) and was subjected to ANOVA analysis using Dunnett's *t*-test [8].

Result

The ethanolic extract of *Verbascum thapsus* shown the presence of Carbohydrates, Flavonoids, Alkaloids, Terpenoids, Tannins, Steroids and Glycosides.

Toxicity study

In toxicity study (limit test) the ethanolic extract *Verbascum thapsus* was shown no signs and symptoms, morbidity and mortality on *Wistar* rats.

Oral Glucose Tolerance Test (OGTT)

In the Oral glucose tolerance test (OGTT) in normal rats **Table 1** showed the blood glucose levels of the Normal control (Normal saline 0.5 ml/kg b.w/p.o), Glibenclamide (10 mg/kg b.w/p.o) and ethanolic extract of *Verbascum thapsus (25*0 mg and 500 mg/kg b.w/p.o) at different time points (0, 30, 60, 120, 150 min) after oral administration of glucose (2 g/kg b.w/p.o). There was a peak increase in the blood glucose at 30 min in all the groups.

Serum lipid profile

In serum profile the elevated cholesterol, triglycerides, VLDL, LDL levels and decreased HDL levels were reported in the diabetic rats. In this study administration of extract of *Verbascum thapsus* (L). **Table 3** showed significantly reduced the elevated cholesterol, triglycerides, VLDL and LDL levels in diabetic rats. Also increased the levels of HDL were observed in diabetic rats.

Histopathology examination of pancreas section, In the diabetic group, decrease in pancreatic islet numbers and size, atrophy and vacuolation, and damage of islets was detected, but these abnormal histological signs dramatically decreased in the group treated with standard and extract (i.e. regeneration of islets).

The plant leaves of *Verbascum thapsus* (L) contains carbohydrates, flavonoids, alkaloids, tannins, terpenoids, steroids and glycosides. Some of these classes of compounds have been implicated in the antidiabetic activity of the plants. Ex: Flavonoids and tannins. The ethanolic extract of *Verbascum thapsus* (L) was shown significant antidiabetic activity when

Table 3: Effect of treatment on Serum profile of different groups in diabetic rat

Group	Serum lipid profile (mg/dl)				
	Total cholesterol	**Triglycerides**	**HDL**	**LDL**	**VLDL**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
I (Normal control)	63.2 ± 2.280	60.4 ± 1.47	33.0 ± 1.040	18.1 ± 2.500	12.1 ± 0.293
II (Diabetic control)	158.0 ± 1.900	144.0 ± 4.87	14.3 ± 0.282	114.0 ± 1.820	28.8 ± 0.973
III (Standard)	73.6 ± 1.130***	63.9 ± 2.19***	26.2 ± 1.500***	34.6 ± 1.230***	12.8 ± 0.438***
IV (Test-I)	92.5 ± 2.030***	95.7 ± 1.42***	20.8 ± 0.801**	52.6 ± 2.520***	19.1 ± 0.283***
V (Test II)	75.1 ± 1.590***	65.3 ± 1.86***	25.8 ± 1.380***	36.2 ± 2.240***	13.1 ± 0.373***
Values are Mean ± SEM; N=6; *P value< 0.01; **p <0.02; ***p < 0.001 *vs.* Diabetic control					

compared to standard drug glibenclamide 10 mg/kg b.w/p.o in alloxan induced diabetic *Wistar* rats.

In toxicity study (limit test) the ethanolic extract *Verbascum thapsus* (L) was shown no signs and symptoms, morbidity and mortality on Wistar rats.

In OGTT there was a peak increase in the blood glucose at 30 min in all the groups. In Glibenclamide and Extract treated groups, there was a decrease in blood glucose level at 180 min when compared to control group.

In Alloxan induced diabetic rats, the extract at doses of 250 and 500 mg/kg b.w/p.o showed a significant reduction in the blood sugar level after 3rd day and 1st day respectively.

At the end of the study the extract at dose of 250 and 500 mg/kg b.w/p.o showed a significant ($p<0.001$) reduction in the blood glucose level comparable with that of glibenclamide (10 mg/kg b.w/p.o) treated group.

In serum lipid profile the elevated cholesterol, triglyceride, VLDL, LDL levels and decreased HDL levels were reported in diabetic rats. In this study administration of extract of *Verbascum thapsus* (L) significantly reduced the elevated cholesterol, triglyceride, VLDL and LDL levels in diabetic rats. Also increased the levels of HDL were observed in diabetic rats. Therefore, this plant extract may be helps in preventing the diabetic associated complications.

From the histopathological studies, it was suggested that β cells destruction by alloxan was inhibited, this might be the primary cause for the antidiabetic activity of the extracts (**Figure 1**). There is no destruction of β cells in normal control group, complete damage of β cells was observed in diabetic control group, and less cellular damage of beta cells was observed in all extract test groups and standard group but better in test-II (500 mg/kg b.w/p.o) when compared to diabetic control group i.e. equal to that of standard drug treated group.

The *Verbascum thapsus* (L) extract (500 mg/kg b.w/p.o) was shown a better significant antidiabetic activity. In Alloxan induced diabetic rats, the extract at dose of 500 mg/kg b.w/p.o showed a significant blood glucose reduction from 1st day of treatment. The damage of β-cells of pancreas in alloxan induced diabetic control rats and regeneration of β-cells by standard rats was observed.

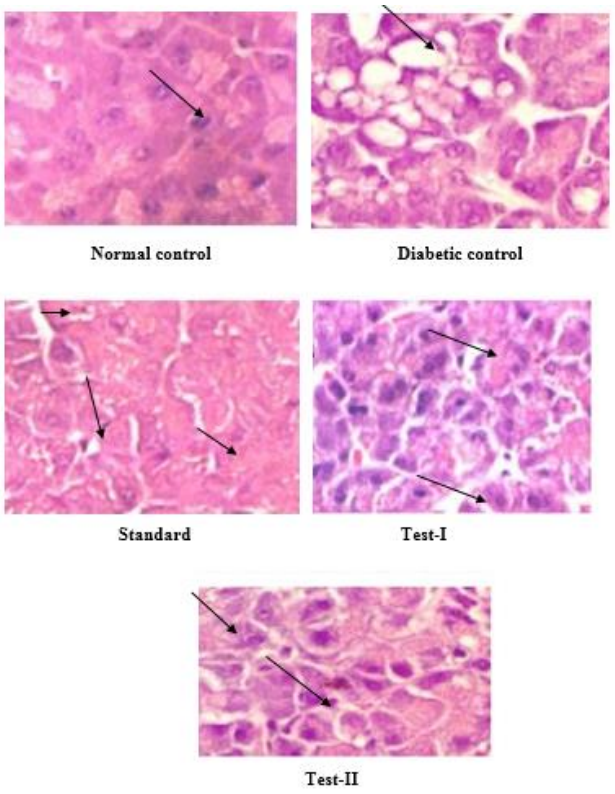

Figure 1: Histopatological changes in rat pancreas

The protection of beta cells was also shown by ethanolic extracts of *Verbascum thapsus* (L). Hence the above discussion revels that ethanolic extract at test-II (500 mg/kg b.w/p.o) is effective and shows similar curative effect as standard (glibenclamide 10 mg/kg b.w/p.o).

Overall the present investigation has shown the presence of active phytochemicals in the ethanolic extract of *Verbascum thapsus* (L) and rich mixture of flavonoids, Tannins and Terpenoids components have significant antidiabetic activity.

Conclusion

It can be concluded that the ethanolic extract of leaves of *Verbascum thapsus* exhibited significant antidiabetic activity via phytochemical (Flavonoids, Alkaloids, Tannins) constituents, antioxidant property of the extract. Histopathological study, β-cells

protective effect in alloxan-induced diabetic rats. The study validates the traditional use and shows a possible beneficial role of *Verbascum thapsus* remedy diabetic mellitus. Further study is required for the evaluation of mechanism of action.

Conflict of Interests

None Declared.

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None Declared.

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