Research Article

Evaluation of Antidiabetic and Antihyperlipidemic Effects of Methanolic Extract of *Verbascum thapsus* in Alloxan-Induced Diabetic Albino Mice

Waheed Khan[1], Muhammad Ajmal Khan[2], Bakhtawar Khan[1*], Aftab Amin[3], Muhsin Ali[4], Awais Farid, Ali Hazrat[5] and Muhammad Yahya[5]

[1]Department of Zoology Hazara University Mansehra, Khyber Pakhtunkhwa, Pakistan; [2]Centre of Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan; [3]School of Chinese Medicine and Department of Biology, Hong Kong Baptist University, Hong Kong, China; [4]Department of Cell Biology Dalian Medical University Liaoning China; [5]Department of Botany, University of Malakand, Chakdara Dir Lowe, Khyber Pakhtunkhwa, Pakistan.

Abstract | A severe endocrine condition known as diabetes mellitus (DM) is defined by excessive blood sugar levels brought on by an absolute or relative lack of insulin synthesis or activity. At present, the existing diabetes mellitus drugs have many adverse reactions. Therefore, it is needed to investigate novel methods to improve DM treatment. Thus plant-based management could be a possible antidiabetic strategy. The objective of the most recent study was to assess Verbascum thapsus's ability to combat hyperlipidemia and diabetes. Mice were given an intraperitoneal injection of alloxan (150 mg kg^{-1}, b.w.) to cause diabetes. There were five groups of mice (n=10): group 1 (normal control) received normal food and water, group 2 (diabetic control) received normal food and clean water, group 3 (diabetic mice) received 200 mg of methanolic plant extract, group 4 (diabetic mice) received 400 mg of methanolic extract, and group 5 (diabetic mice) received 10 mg of the medication Glibenclamide for 28 days. Glucose was measured four times and after 28 days, blood samples were collected to measure the lipid profile. The result showed that methanolic extract of *V. thapsus* significantly (P>0.05) reduced the blood glucose level, TC, TG, LDL, increase HDL and body weight at 400mg kg^{-1} compared to 200 mg and 10 mg of the standard drug after 28 days of treatment. The results of our study suggested that methanolic extract of *V. thapsus* have potent anti-diabetic activity, with comparatively less toxicity for its use in ethno-medicine for diabetes management.

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***Correspondence** | Bakhtawar Khan, Department of Zoology Hazara University Mansehra, Khyber Pakhtunkhwa, Pakistan; **Email:** bakhtawardir161@gmail.com
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Introduction

High blood glucose levels caused by erratic or inadequate insulin secretion are a hallmark of diabetes mellitus (DM), a chronic metabolic illness with many etiologies (Piero *et al.*, 2015). The prevalence of DM is alarmingly rising around the globe. By 2025, researchers project that the prevalence of DM

would rise by 64%, affecting 53.1 million people (Rowley and Bezold, 2000). Type I diabetes is caused by the autoimmune death of pancreatic beta-cells, which results in a complete lack of insulin secretion, as well as by several hereditary and environmental factors (obesity, aging, etc) (American Diabetes Association, 2012). At the onset of hyperglycemia patients typically experience increased urination (polyuria), thirstiness (polydipsia), and incessant appetite (polyphagia), Retinopathy, nephropathy, and neuropathy (Piero *et al*., 2012). Persistent low levels of glucose (hypoglycemia) abrogate fat, protein, and lipid metabolism (Piero *et al*., 2012).

The conventional treatments of DM are often costly, unobtainable and have several adverse health effects (Murugi *et al*., 2012). For example, the utilization of insulin is often associated with low efficacy, a shorter shelf life, over-prescription and resistance. Other drugs such as sulfonylureas and biguanides have been linked to increased body weight (Mukundi *et al*. 2015). Therefore, there is a need to develop high efficacy, low cost, and easily available medicines for the treatment of DM.

Traditional medicinal practices are gaining substantial recognition from mainstream health administrators, global medicinal investigators, and training organizations. According to the World Health Organization, 80% of people in underdeveloped countries utilize traditional medicines because they are generally affordable, safe, effective, and dependable (Surendran *et al*., 2011; Musila *et al*., 2002). Herbal remedies contain countless varieties of bioactive elements that are used by researchers for potential medicinal uses (Mahmood and Mahmood, 2012). For example, Metformin is a highly effectively drug traditionally used for the treatment of DM (Piero *et al*., 2015). It has been reported that more than 800 traditional plants had anti-diabetic properties (Piero *et al*., 2015; Ibrar *et al*., 2014). Certain plants are confirmed to support the renewal of β-cells and promoting the activation of insulin receptors (Pandey *et al*., 2011; Karau *et al*., 2012). Ginseng is used in traditional Chinese medicine for the management of DM (Piero *et al*., 2015). The local flora of Pakistan contains a variety of plants that have potential application in the management of different diseases (Islam *et al*., 2006; Farooq *et al*., 2012).

V. thapsus is a common plant used in traditional medicine. It is widely distributed in the Himalayas (Asia), Europe and North America (Ansari and Daehler, 2000; Hoshovsky, 1988). In Pakistan, it is found in different areas of Khyber Pakhtunkhwa and Kashmir (Murad *et al*., 2011; Shinwari and Gilani, 2003). Previously it is used for the treatment of pulmonary diseases (Shinwari and Gilani, 2003). The leaf has been used as an expectorant and in the treatment of sore throat, bronchitis, and hemorrhoids (Tatli and Akdemir, 2006). The Zuni tribe (western New Mexico) has traditionally used the roots of the plant as covering for rashes and various other skin ailments (Haughton, 1978). The flowers of the plant have applications in the production of hair dyes (Ito *et al*., 2007). The Current study aims to scientifically evaluate hyperglycemia and hyperlipidemia effects of *V. thapsus* extracts in Alloxan-induced diabetic albino mice.

Materials and Methods

Area of study

The National Veterinary Laboratory in Islamabad, Pakistan, and the Department of Zoology at Hazara University in Mansehra, Pakistan, both participated in this study.

Plant collection

The plant materials were collected according to the ethnobotanical guidelines issued by various localities in the district of Mansehra, Khyber Pakhtunkhwa (KP), Pakistan and stored in herbarium of department of botany Hazara University, Mansehra, KP, Pakistan under the voucher specimen No. 4984.

Preparation of extract

The harvested leaves of the plant were dried in a shaded area away from immediate sun shine for one week and pounded into a rough face powder using an electrical grinder. The powder was soaked in 100% methanol (Sigma Aldrich) for 48 hours followed by incessantly shaking for 24 hours. The plant solution was clarified with novel Whitman paper and vaporize using an evaporator machine (Rota vapor R-3) at 50 °C until a sticky dense liquid was achieved, and processed material was kept at 4°C for future use.

Phytochemical analysis of Verbascum thapsus

Qualitative pre-liminary phytochemical analysis was performed on the methanolic extract of *V. thapsus* for presence of chemical constituents such

as alkaloids, tannins, flavonoid, carbohydrates, phenolic compounds and saponins by using standard protocols as described before (Jones and Kinghorn, 2006; Pandey and Tripathi, 2014). These chemical constituents were identified by characteristic color change.

Acute toxicity testing

Toxicity of the extract was tested according to the protocol described before with some modifications (Khaleel, 2020). Briefly, mice were kept on fast overnight and then extract were administered at dose of 1000mgkg^{-1}. b.w. The mice were kept under observation for 24 hrs for behavioral and neurological changes. The mice were observed for 14 days for any toxic symptoms. Some guidelines were set that if mortality is observed in 1 or 2 animals then this dose was considered toxic and if no death occurred then this dose was assigned as non-toxic.

Experimental animals

Fifty, 8-10 weeks old, healthy male mice (BALB/C), weighing 30-40 g were bought from the National Institute of Health (NIH), Islamabad, and acclimatized for one week at the animal house. The mice were provided normal food and water, *ad libitum*.

Standard drug (Glibenclamide)

Standard drug (Glibenclamide) tablets (10 mg) were bought from Sanofi-Aventis Pakistan limited for a positive control in the experiments. The pills were crushed into satisfactory residues and liquefied in refined water to make a solution.

Induction of diabetes

For the diabetes induction, the mice were starved and only allowed to drink water. A single dose (150 mgkg^{-1}) of Alloxan monohydrate (Sigma Aldrich, USA) was freshly prepared in phosphate buffer saline (PBS) for intra-peritoneal injection. The blood glucose levels of the mice were measured 72 hours after Alloxan injection. Mice that had a fasting blood glucose level above 200 mg/dl were considered diabetic, while mice that had a blood glucose level of less than 200 mg/dl were exclude from the study.

Experimental design

A total of 50 strong male mice were selected for the experiment and separated into five clusters: Each set consisting of 10 mice.

Group I-Normal control: Given normal diet and sterilized water throughout the experiment.

Group II- Diabetic control: fed standard food and normal water.

Group III – Extract treated: Treated with *V. thapsus* plant extract (200 mgkg^{-1}) for 28 days.

Group IV – Extract treated: Treated with *V. thapsus* extract (400 mgkg^{-1}) for 28 days.

Group V-standard drug treated: Treated with commercially available medicine Glibenclamide (10 mgkg^{-1}) for 28 days.

Bodyweight counting

The body weight of the mice was measured every week during the entire experiment period and the variations were documented.

Blood glucose determination

Blood samples were extracted from the tail of the mice. The blood glucose concentration was measured by Glucometer, using glucose strips (On-Call Extra). Blood glucose concentrations were recorded and expressed in mg/dl.

Lipid profile determination

Mice are sacrificed after anesthesia and blood is removed directly from the heart by a sterile syringe. Plasma is allowed to stand at room temperature for 1 h for the hemocoat, then spinning at 5,000 rpm for 15 min and the supernatant (serum) is used for lipid analysis. Serum concentrations of high-density lipoprotein (HDL), low-density lipoprotein (LDL) was measured using a reporting protocol with some modifications (Friede-wald, 1972). Total cholesterol (TC), and triglycerides (TG) was analyzed utilizing a technique as already established (Trinder, 1969).

Statistical analysis

The data in this study was expressed as mean ± SD from triplicate experiments. Data was analyzed by one-way ANOVA (analysis of variance) using Graph Pad Prism 5.

Results and Discussion

Preliminary phytochemical screening

In this study the phytochemical screening of *V. Thapsus* showed the occurrence of many secondary metabolites that are concise in the Table 1. Historically, plant-based medicines have been extensively used worldwide for the treatment of DM

(Choudhury *et al*., 2018). Therefore, researchers have been trying to develop plants-based medicines for DM management, as they have little side effects, high efficacy and relatively cheap and easily assessable (Khan *et al*., 2020). Screening of plant extract is important for novel drug search. In accordance to the present study (Sutar and Garai, 2010) reported the similar phytochemical constituents in *V. thapsus*. The qualitatively phytochemical screening of *V. thapsus* showed that this plant has abundant biologically active compounds which perform significant curing role in many diseases.

Table 1: *Test results of different metabolites.*

Metabolites	Results
Tannins	++
Saponins	++
Proteins and amino acids	--
Alkaloids	++
Terpenoids	++
Flavonoids	++
Coumarins	--
Glycosides	++

Notes: ++ = *Test Positive;* -- = *Test Negative.*

Acute toxicity studies

To check the safer and non-toxic dose of *V. thapsus*, mice were treated with 1000 mgkg^{-1}.b.w and kept under observation for behavioral and neurological symptoms. No behavioral and neurological signs and symptoms were observed in all tested mice. No mortalities were observed at 1000 mgkg^{-1} b.w drug dose during 14 days of treatment. Hence it was found that *V. thapsus* is safe and have no toxic effects on mice. Therefore, in current study different doses (200,400/kg. b.w) were selected for treatment purpose.

Week wise effect of the methanolic extract of V. thapsus on glucose level

Results shows that mice treated with 400 mgkg^{-1}of the *V. thapsus* methanolic extract showed significant ($P<0.05$) reduction in fasting blood glucose concentrations relative to the groups that were treated with glibenclamide, (10 mgkg^{-1}) and normal control group (Table 2). Our findings revealed that methanolic extracts of *V. thapsus* have strongly antihyperglycemic activities at two different concentrations (200 and 400 mgkg^{-1}). Likewise, a previous study reported that methanolic leaf extract of *Stephania japonica* (200, 350 mgkg^{-1}) is effective in reducing blood glucose concentrations in Alloxan induced diabetic rats in four weeks of treatment (Zehad *et al*., 2017).

Similarly, another study reported that an aqueous leaf extract of *C. hirsutus* treatment for four weeks was effective at the high concentration (1000 mgkg^{-1}) ($P < 0.01$) in reducing blood glucose concentrations (Badole *et al*., 2006). This result is comparable to our findings even though the effective dose of *C. hirsutus* (1000 mgkg^{-1}) is significantly greater than the effective dose identified for *V. thapsus* (400 mgkg^{-1}), suggested that *V. thapsus* is also more effective. Another study regarding the administration of Phyllanthus amarus ethanolic extract (400 mgkg^{-1} b.w for 7 weeks and three days) also have similar results as in our study (Shetti *et al*., 2012). The prolonged management period with *P. amarus* may be considered more effective than *V. thapsus*, however this hypothesis needs to be further verified in the future.

Measurement of mice body weights

A positive correlation is present in gain of body weight with plant extract treatment during DM. In current study a significant upsurge in weight of the body was observed in treated groups, while a significant reduction in body weight was seen in the diabetic control group. The oral administration of *V. thapsus* extract was noticed to rise the body weight of the mice, 25.5±4.4 to 31.5±6.7 in the 200 mgkg^{-1} treatment group, 26±2.1 to 36±3.3 in the 400 mgkg^{-1}

Table 2: *V. thapsus*'methanolic leaf extract and glibenclamide effect on fasting blood glucose levels after four weeks in Alloxan induced diabetic mice. Values are expressed as Mean ± SD, (n= 10), *$P < 0.05$.

	Blood glucose levels (mgdL^{-1}) per week				
Treatment groups	**Day 1**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Normal Control (NC)	118.8±20.0	90.4±12.2	108.6±14.8	106.5±17.4	109.9±13.0
Diabetic Control (DC)	284.7±132.9	335.7±120.0	373.4±102.4	366.5±75.5	372±88.6
V. thapsus extract 200 mgkg^{-1}(T1)	334.3±104.2	293.4±96.7	239.4±133.7	232.5±153.3*	225.6±118.2*
V. thapsus extract 400 mgkg^{-1} (T2)	308.6±175.3	187.5±91.3*	178.5±150.6*	172.7±84.0*	164.5±84.8*
Glibenclamide10 mgkg^{-1} (SD)	297.8±69.0	273.4±58.7	220±75.6*	191.5±54.5*	186.2±64.2*

treatment group, respectively, relative to the diabetic control group (P<0.05) Table 3. It has been established in previous studies that the increase in body weight of diabetic mice might be expected to the enhanced production of insulin (Kirana and Srinivasan, 2010; Solomon *et al*., 1999) and build-up of protein. Other similar studies involving *Ficus bengalensis* and *foenumgreacum* plant treatments demonstrated similar result in increasing body weight of Alloxan induced diabetic rats (Srinivasulu *et al*., 2015; Schwechter *et al*., 2003; Mowla, 2009). In a study regarding the oral administration of *C. hirsutus* aqueous leaf extract for four weeks showed significant increases in body weight, suggesting positive influence of the treatment (Shetti *et al*., 2012).

Measurements of lipid profile

Lipids play an important role in the pathogenesis of DM as altered lipid metabolism leads to elevated cholesterol levels. In the current study, elevated cholesterol levels were observed in diabetic control mice treated with the extract experienced a gradual decrease in cholesterol levels Table 4 shows the effects of glibenclamide and methanol extracts of *V. thapsus* on serum TG, HDL-C, LDL-C and TC TG levels were significantly (p<0.05) higher in diabetic control mice A diabetic mouse showed a significant reduction in TG levels when he was treated for 4 weeks with different doses of *V. thapsus* (200, 400 mgkg^{-1}). TC and LDL levels. After administration of different doses of *V. thapsus* and glibenclamide (10 mg) significantly decreased (p < 0.05) compared to the diabetic group, which showed this. Methanol extract of *V. thapsus* Effective in normalizing cholesterol levels in alloxan-induced diabetics mouse. Lipids performed a vital role in causing of diabetes (Sharma *et al*., 2006). The main complications of high cholesterol are hypercholesterolemia and Hypertriglyceridemia in diabetes (Al-Shamaony *et al*., 1994) In the present study, we observed a decrease in *V. thapsus* leaf extract. Blood cholesterol levels at doses of 400 and 200 mgkg-1 body weight). Studies show that Nigella Sativa Leaf extract reduced cholesterol levels in STZ-induced diabetic rats (350 mgkg-1) for 3 weeks. Treatment compared to diabetic control mice (Al-Logmani, 2009). In our current study, diabetic mice were treated with *V. thapsus* extracts (200 and 400 mgkg-1) for 28 days showed a reduction in low concentration levels Lipoprotein Cholesterol (LDL), Enhanced High Density Lipoprotein (HDL) (TC) and triacylglycerols (TG) were significantly regularized (P>0.05) in accordance with the previously published work (Khan *et al*., 2021). The reduction in cholesterol levels after *V. thapsus* extract treatment may be due to the superior action of certain enzymes, such as the lecithin-cholesterol acyltransferase enzyme, which controls blood lipid levels and converts fatty acids into their storage form (triglycerides).

Table 3: *Methanolic leaf extract of V. thapsus and glibenclamide effects on the mice body weight. Data are expressed as Mean ± SD, (n= 10), *P < 0.05.*

Different doses effects on bodyweight of mice (gm) per week					
Treatment groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal Control	36±3.4	34.5±2.9	35.5±1.7	35±2.5	35±1.7
Diabetic Control	37.5±3.5	35.5±6.2	28±8.3	23.5±3.8	20.5±2.5
V. thapsus extract 200 mgkg^{-1}	25.5±4.4	26±4.6	29±5.2	31±2.1*	31.5±6.7*
V. thapsus extract 400 mgkg^{-1}	26±2.1	27±3.5	31±2.1*	34.5±2.8*	36±3.3*
Glibenclamide 10 mgkg^{-1}	27.5±5.4	28±4.2	29±2.1	31±3.9	32.5±5 .4

Table 4: *Effect of different doses of methanol extracts of V thapsus and glibenclamide on the lipid profile of diabetic mice after 4 weeks of treatment. The data are represented as Mean ± SD, (n= 10), *P < 0.05.*

Lipid profile				
Treatment groups	**T. G**	**HDL**	**LDL**	**Total cholesterol (TC)**
Normal control (NC)	73.59±11.64	45±2.5	56.87±4.00	116.83±11.20
Diabetic Control (DC)	129.63±21.53	37.35±2.58	98.73±9.5	145.13±7.94
V. thapsus extract 200 mgkg^{-1}	121.53±15.01	38.67±3.51	93±2.7	139.77±20.61
V. thapsus extract 400 mgkg^{-1}	99.54±14.01*	42.86±2.42*	75.67±3.64*	126.45±15.61*
Glibenclamide dose 10mgkg^{-1}	118.56±15.65	39.32±2.23	88.56±2.43	136.55±16.73

There is insulin deficiency also contributes to cholesterol deposition, as insulin inhibits transport of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), which leads to deprivation of cholesterol-rich LDL elements (Kirana and Srinivasan, 2010). The positive results from this study strongly advocate further biochemical and pharmacological studies so that the bioactive components and their respective mechanisms of action may be ascertained. There is an abundance of natural materials that may potentially be used to manage and treat various human diseases. This study has demonstrated that the *V. thapsus* plant has great implications as a medicine for the treatment of DM.

Conclusions and Recommendations

The methanolic extract of Verbascum thapsus's ability to combat hyperlipidemia and diabetes is attributed to the presence of various essential phytochemicals. The administration of methanolic extract orally to the mice was found to reduce the blood glucose level, positive correlation is present in gain of body weight with treatment and improved lipid profiles. The current study results warrant the further investigation on Verbascum thapsus to further explored its antidiabetic potential by including human subjects.

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Novelty Statement

This is the first study to evaluate hyperglycemia and hyperlipidemia effects of methanolic extract of V. thapsus extracts in Alloxan-induced diabetic albino mice, collected from various localities in the district of Mansehra, Khyber Pakhtunkhwa (KP).

Author's Contribution

Acquisition of the data, Development of methodology, Writing, review, by Waheed Khan, Muhammad Ajmal Khan. Conception and design, review, and/or revision, Bakhtawar Khan. Writing, Review the final manuscript, Aftab Amin, Ali Hazarat. Acquisition of the data and analysis, Muhsin Ali, Awais Farid, Muhammad Yahya.

Conflict of interest

The authors have declared no conflict of interest.

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