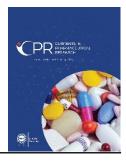
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# Evaluation of the Antidiabetic Activity of *Asphodelus Tenuifolius* in Normal and Alloxan-induced Diabetic Rats

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## ABSTRACT

Asphodelus tenuifolius is traditionally used for the treatment of diabetes mellitus (DM). The current study aims to investigate the antidiabetic activity of Asphodelus tenuifolius in normal and alloxan-induced diabetic rats. Ethanolic and aqueous extracts of whole Asphodelus tenuifolius plant were prepared by using the maceration process. DM was induced by a single intraperitoneal injection of alloxan monohydrate (140 mg/kg b.w) in rats. Glibenclamide was used as the reference drug. In an acute study, ethanolic extract of Asphodelus tenuifolius (ATEE) and aqueous extract of Asphodelus tenuifolius (ATAqE) were administered in 200 and 400 mg/kg doses to normal and alloxan-induced diabetic rats. Both extracts (ATEE and ATAqE) significantly lowered the blood glucose level of both normal and diabetic treated rats in a concentration dependent fashion. However, ATEE produced prominent results at the dose of 400 mg/kg. In a fourteen-day study, ATEE considerably decreased the blood glucose level of alloxanized rats. The results were similar to the reference drug, that is, glibenclamide. In the prolonged study, the effects of ATEE on liver enzymes and hematological parameters of diabetic rats were also studied. Hb level and platelet count was increased in ATEE-treated diabetic rats as compared to diabetic control. However, it did not affect other hematological parameters. ATEE significantly decreased the ALP level as compared to diabetic control. Although, the test extract did not significantly alter the SGOT and SGPT levels. Further, the phytochemical testing of ATEE revealed the presence of alkaloids, flavonoids, tannins, and terpenoids. It was concluded that Asphodeleus tenifolius possesses antidiabetic activity. More comprehensive studies are needed in the future to explicate the mechanism of action and to characterize the phyto-components of this plant.

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Keywords: Asphodelus tenuifolius, alloxan, diabetes mellitus (DM), hematology, liver enzymes

#### **1. INTRODUCTION**

Diabetes mellitus (DM) is characterized by an increase in blood glucose level, as well as disturbed carbohydrate, fat, and protein metabolism, secondary to the lack of insulin. Dyslipidemia is reportedly involved in micro- and macro -vascular complications of DM, accounting for morbidity and mortality [1]. Chronic hyperglycemia has been demonstrated as the main characteristic of diabetes causing glycation proteins with subsequent defects in eyes, kidneys, arteries, and nerves. The World Health Organization (WHO) reports that the number of diabetic patients would increase up to three hundred million or more by 2025 [2]. The currently available antidiabetic drugs are expensive for a common man and associated with adverse effects. Therefore, there is a need to search for newer, costeffective, antidiabetic agents with fewer adverse effects [3]. Plants have been a source of drugs since ancient times. Asphodelus tenuifolius Cav. belongs to the family Asphodelaceae. It is commonly known as piazi in Pakistan [4]. It is commonly found in Cholistan desert in District Bahawalpur and in Tehsil Chestain, District Bahawalnagar. The length of the plant is 15-50 cm. It has cylindrical leaves having white flowers with a pink colored central stripe [5]. Its leaves are linear and simple. There are a number of seeds which are triangular in shape and blackish gray in colour, as shown in Figure 1.



Figure 1. Asphodelus tenuifolius Can. Whole Plant

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The whole plant is used traditionally for medicinal purposes, such as diuretic, anti-inflammatory, skin diseases, constipation, swelling, hypertension, and piles [ $\underline{6}$ ,  $\underline{7}$ ]. Pharmacological studies discovered that the plant has antibacterial and anti-oxidant activities [ $\underline{8}$ ], as well as hypotensive and diuretic properties [ $\underline{5}$ ]. In the current study, the antidiabetic activity of *Asphodelus tenuifolius Cav.* was evaluated in alloxan-induced diabetic rats.

### 2. MATERIALS AND METHODS

### 2.1. Chemicals and Drugs

Chemicals of standard grade were used in the study. These chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA). The chemicals and drugs used included alloxan monohydrate, potassium oxalate, sodium fluoride, glibenclamide, ethanol, and glucose.

## 2.2. Animals

Sprague-Dawley rats of either sex of body weight (200-220 g) were used in the study. Animals were kept at the temperature  $25\pm2^{\circ}C$  and housed in the animal house of Lahore College of Pharmaceutical Sciences, Lahore, Pakistan. Prior to and throughout the experiment, rats were provided with a standard laboratory pellet diet and unrestricted access to water. Following randomization, the rats were allowed to adapt to different groups for a period of 2-3 days within a new environment before commencing the experiment.

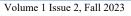
### 2.3. Plant Material

The selected plant was collected from District Bahawalnagar, Punjab, Pakistan. The plant was identified and authenticated by a taxonomist. A voucher specimen (Bot.3480) was deposited at the herbarium of the GC University, Lahore to serve as a reference for the future. The plant material was air-dried in shade and then processed into coarse powder using a Chinese herbal grinder.

## 2.4. Preparation of Extracts

With the help of electric grinder, the plant material was powdered and then soaked into ethanol and distilled water separately at ordinary laboratory temperature with occasional shaking for a period of 72 h. Then, it was filtered using muslin cloth and finally through Whatman paper (grade 1). The whole process was repeated with residue with fresh solvents. For

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ethanolic extract, the filtrate was evaporated using rotary evaporator. For aqueous extract, the filtrate was evaporated at room temperature. The crude extracts were preserved in the refrigerator  $[\underline{5}]$ .

### 2.5. Preparation and Administration of Drugs

Using the weighing balance, the quantity of the extracts and drugs for each animal was calculated on the basis of weight. Both extracts were dissolved in distilled water or normal saline. Then, each suspension was administered orally to each animal according to body weight by using a disposable syringe.

### 2.6. Induction of Experimental Diabetes

The rats received an intraperitoneal injection of alloxan monohydrate dissolved in normal saline, administered at a dosage of 140 mg/kg of body weight. After seventy-two hours, rats with moderate DM having medium level hyperglycemia were used for the experiment [9]. Fasting glucose was measured. Animals with greater than 300 mg/dl of blood glucose level were excluded from the study. The selected animals were divided into five groups (n=5) [10]. Blood glucose level was measured by using glucometer (Optium Xceed, Abbot Laboratories, USA).

# **2.7.** Hypoglycemic Activity of *Asphodelus tenuifolius* (ATEE) Extracts in Normal Rats

The chosen animals were sorted into five groups, each comprising five rats. Following an overnight fasting period, the rats were divided into different groups. Group 1 served as the control (untreated) group and received a dose of 0.5ml/100g of the vehicle. Group 2 and Group 3 were administered *Asphodelus tenuifolius* (ATEE) at doses of 200 and 400mg/kg, respectively. Further, Group 4 and Group 5 were treated with *Asphodelus tenuifolius* (ATAqE) in 200 and 400mg/kg doses, respectively. Blood samples were collected from the tail tip at zero, two, four, and six hours after vehicle and drug administration.

# 2.8. Screening of *Asphodelus tenuifolius* (ATAqE) Extracts for Antihyperglycemic Activity in Diabetic Rats

The diabetic rats were divided into Group 1: Diabetic control that received a single dose of 0.5ml/100g of vehicle. Group 2 and 3 were treated with ATEE at doses of 200 and 400mg/kg. Group 4 and 5 were treated with ATAqE at two dose level of 200 and 400mg/kg. Samples of blood were

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collected for assessment of blood glucose level obtained from the tail tip at zero, two, four and six hours after treatment.

# **2.9.** Prolonged Antidiabetic Activity of Ethanolic Extract of *Asphodelus tenuifolius* (ATAqE)

In the acute study, ethanolic extract produced comparatively significant antihyperglycemic effects, therefore, prolonged antidiabetic activity was performed by using ATEE. Rats with diabetes were divided into three groups (n=5). Group 1 comprised diabetic control that received vehicle. Group 2 comprised diabetic rats treated with ATEE at a dose of 400mg/kg of body weight. Group 3 comprised diabetic rats that received standard glibenclimide at a dose of 5mg/kg of body weight [9] for fourteen consecutive days. All drugs were administered once daily.

# **2.10.** Effect of Ethanolic Extract of *Asphodelus tenuifolius* on Body Weight of Diabetic Rats

During the above experiment, body weight of all rats in three groups were assessed on day 0, 7, and 14 [ $\underline{10}$ ].

# **2.11.** Effect of Ethanolic Extract of *Asphodelus tenuifolius* on Liver Enzymes of Diabetic Rats

Serum SGPT, SGOT, and ALP levels were elevated in experimentallyinduced DM. The objective was to explore the impact of ATEE on liver enzymes. On the 14<sup>th</sup> day of the experiment, blood samples were collected from the tail vein of the rats of three groups to assess the serum levels of SGPT, SGOT, and ALP.

# **2.12.** Effect of Ethanolic Extract of *Asphodelus tenuifolius* on Hematological Parameters of Diabetic Rats

On the 14<sup>th</sup> day of the experiment, blood samples were collected from the tail vein of rats of three groups to assess the hematological parameters.

# 2.13. Phytochemical Testing of the Ethanolic Extract of Asphodelus tenuifolius

Ethanolic extract of *Asphodelus tenuifolius* was assessed to determine the phytochemical constituents present in it by using the following standard procedures [<u>11</u>].

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**2.13.1. Test for Alkaloids.** Few ml of filtrate solution (ethanolic extract) was taken in a test tube and 1ml of Dragendroff's test reagent was added. The appearance of yellow color indicated the presence of alkaloids.

**2.13.2. TLC Plate Method.** Ethanolic extract was spotted on TLC plate and sprayed with Dragendroff's test. Bright yellow spots appeared on TLC plate.

**2.13.3. Test for Flavonoids.** In 1ml of ethanolic extract, 1ml of 10% solution of lead acetate was added with subsequent appearance of yellow precipitates.

**2.13.4. TLC Plate Method.** The ethanolic extract of the plant was spotted on TLC plate for the determination of flavonoids. The plate was then sprayed with aluminum chloride. Bright yellow florescence was produced.

**2.13.5. Test for Terpenes.** In 2ml of extract, the same volume of distilled water and a few ml of ferric chloride solution were added. Green precipitates appeared to confirm the presence of terpenes.

**2.13.6. TLC Plate Method.** TLC plate was spotted with the ethanolic extract of the plant. Ceric sulphate solution was then sprayed on the plate. Dark brown spots were produced.

**2.13.7. Test for Phenol and Sugar.** Ethanolic extract of plant was spotted on TLC plate. The plate was then sprayed with the solution of anisaldehyde. Red colored spots were produced.

**2.14.8. Test for Saponins.** Ethanolic plant extract of saponins was added to the test tube with an equal quantity of water. The test tube was shaken vigorously for about 5 mins. Then, it was allowed to stand for 30 mins. The appearance of honeycomb froth was indicative of the presence of saponins.

#### 2.15. Statistical Analysis

The data were presented as mean  $\pm$  standard error of mean (SEM) and analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni posttest. P < 0.05 was considered as significant.

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### **3. RESULTS**

### 3.1. Hypoglycemic Activity of Asphodelus tenuifolius in Normal Rats

Ethanolic extract of *Asphodelus tenuifolius* significantly (p < 0.001) reduced the blood glucose level in normal rats at the doses of 200 and 400mg/kg in a time and concentration dependent manner. Aqueous extract also reduced the blood glucose level and produced significant results at the fourth and sixth hour but non-significant results at 2 hours of post-treatment (Table 1, Figure 2).

# **3.2.** Screening of Ethanolic and Aqueous Extracts of *Asphodelus tenuifolius* for Hypoglycemic Activity in Diabetic Rats

Ethanolic extract of *Asphodelus tenuifolius* produced a very significant (p < 0.001) reduction in blood glucose level of diabetic rats. Aqueous extract also produced significant (p < 0.001) reduction in blood glucose level at one dose, that is, 200mg/kg (Table 2, Figure 3).

# **3.3.** Effect of Ethanolic Extract of *Asphodelus tenuifolius* on Blood Glucose Level of Diabetic Rats

Ethanolic extract of *Asphodelus tenuifolius* significantly (p < 0.01) reduced the blood glucose level of diabetic treated rats during the fourteenday study. The results were comparable with glibenclimide which also significantly reduced the blood glucose level (Table 3, Fig 4).

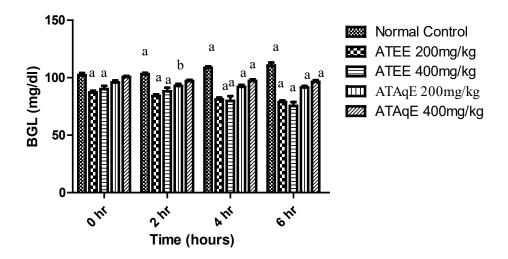
Table 1. Blood Glucose Level (mg/dl) of Normal Rats Treated	d with
Ethanolic and Aqueous Extracts (200 mg/kg) and (400 mg/kg) o	f Body
Weight	

Time (hours)	Normal Control	ATEE 200mg/kg	ATEE 400mg/kg	ATAqE 200mg/kg	ATAqE 400mg/kg
0	102 ±4.207	$87 \pm \! 3.807^a$	$94{\pm}6.97^{\rm a}$	$95.8 {\pm} 3.83^{ns}$	100.6±2.073 <sup>ns</sup>
2	103 ±2.915	$84\pm\!3.464^a$	82.2 ±9.28 <sup>a</sup>	92.8±4.417 <sup>b</sup>	97±2.549 <sup>ns</sup>
4	108.6 ±3.04	$81\pm\!\!3.464^a$	79.8 ±9.17ª	92±4.06 <sup>a</sup>	97.2±3.114 <sup>a</sup>
6	110.6 ±5.72	$78 \pm 2.509^{a}$	75.4 ±7.56 <sup>a</sup>	91.8±3.271ª	96.4±3.286ª





Data are shown as Mean ±SEM (n=5) produced significant result at (p < 0.01) vs normal control, where (<sup>a</sup>) = (p < 0.001), (<sup>b</sup>) = (p < 0.01), and (<sup>ns</sup>) = non-significant vs. control



**Figure 2.** Blood glucose level of healthy rats administered with ethanolic and aqueous extracts at doses of 200 and 400 mg/kg, respectively where (<sup>a</sup>) = (p < 0.001), (<sup>b</sup>) = (p < 0.01) compared to the normal control ATEE = *Asphodelus tenuifolius* ethanolic extract, ATAqE = *Asphodelus tenuifolius* aqueous extract.

Table 2. Level of Blood	Glucose (mg/dl)	in	Diabetic	Rats	Treated	with
Aqueous Extracts						

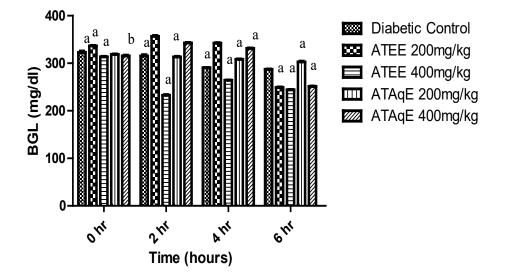
Time (hours)	Diabetic Control	ATEE 200 mg/kg	ATEE 400 mg/kg	ATAqE 200 mg/kg	ATAqE 400 mg/kg
0	324.4	336.4	313.6	318.4	316.25
0	$\pm 7.987$	$\pm 2.701$	$\pm 2.073^{a}$	$\pm 2.701$	$\pm 3.5^{b}$
2	315	383.8	232.6	313.2	343.75
Δ	$\pm 5.805$	$\pm 1.923^{ns}$	$\pm 2.701^{a}$	$\pm 3.114^{a}$	$\pm 2.62$ ns
4	290.6	342.2	264	308	331.75
4	$\pm 1.516$	$\pm 1.92^{a}$	$\pm 1.581$ <sup>a</sup>	$\pm 1.923^{a}$	±1.70 <sup>a</sup>
6	287	248.8	244	302.8	251
0	$\pm 1.581$	$\pm 3.193^{a}$	$\pm 1.581^{a}$	±3.19 <sup>a</sup>	±1.581 a

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Data are shown as Mean  $\pm$  SEM produced significant results at (p < 0.001) where (<sup>a</sup>) = (p < 0.001), (<sup>b</sup>) = (p < 0.01) and (<sup>ns</sup>) = non-significant vs. control



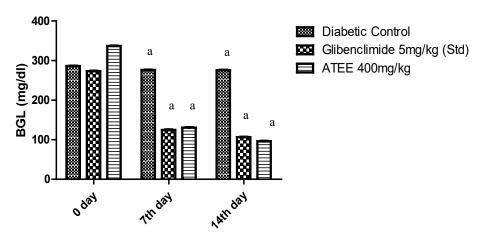
**Figure 3.** Level of blood glucose in diabetic rats treated with ethanolic and aqueous extracts at doses of 200 and 400 mg/kg produced significant results at (p < 0.001) and non-significant at (p > 0.05), where  $\binom{a}{b} = (p < 0.001)$ ,  $\binom{b}{b} = (p < 0.01)$ . ATEE = Asphodelus tenuifolius ethanolic extract, ATAqE = Asphodelus tenuifolius aqueous extract

Time (dava)	Diabetic	Glibenclimide	ATEE
Time (days)	Control	(5mg/kg)	(400mg/kg)
0	$286 \pm 2.286$	$273 \pm 1.923^{ns}$	$336.8 \pm 2.3874^{ns}$
7 <sup>th</sup>	$277.2 \pm 2.387$	$124.6 \pm 2.408^{a}$	130.4 ±2.7018 <sup>a</sup>
$14^{\text{th}}$	$275.6 \pm 2.0736$	$106.4 \pm 2.073^{a}$	$95.8 \pm \! 2.3874^a$

**Table 3**. Blood Glucose Level (mg/dl) of Diabetic Control, Glibenclimide, and Ethanolic Extract Treated Diabetic Rats

Values are expressed as Mean  $\pm$  SEM (*n*=5), where (<sup>a</sup>) = (*p* < 0.001) as compared to diabetic control.

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**Figure 6.** Blood glucose level of diabetic rats treated with ethanolic extract at a dose of 400 mg/kg and glibenclimide at a dose of 5 mg/kg for fourteen days produced significant results at (p < 0.001) where (<sup>a</sup>) = (p < 0.001), ATEE = *Asphodelus tenuifolius* ethanolic extract

# **3.4 Effect of Ethanolic Extract of** *Asphodelus tenuifolius* on Body Weights of Diabetic Rats

Ethanolic extract of *Asphodelus tenuifolius* did not prevent weight loss during the fourteen-day treatment and the weight of rats treated with the extract significantly decreased (Table 4).

# **3.5** Effect of Ethanolic Extract of *Asphodelus tenuifolius* on Liver Enzymes of Diabetic Rats

Ethanolic extract did alter the levels of liver enzymes (SGOT and SGPT) except ALP, as compared to control. However, treatment with glibenclimide significantly reduced all the measured liver enzymes levels (Table 5).

# **3.6 Effect of Ethanolic Extract of** *Asphodelus tenuifolius* on Hematological Parameters of Diabetic Rats

With the treatment of ethanolic extract, monocytes and esinophils remained within the normal range and there was no significant change. Treatment with ethanolic extract and glibenclamide platelet count exhibited a notable increase as compared to the diabetic control group. Furthermore, the Hb level also showed an increase as compared to the diabetic control group (Table 6).

# **3.7.** Phytochemical Constituents in Ethanolic Extract of Asphodelus tenuifolius

Phytochemical testing of ethanolic extract indicated the presence of alkaloids, flavonoids, terpenes, sugars, phenols, and tannins, while saponins were found to be absent, as shown in Table 7.

**Table 4.** Weight of Diabetic Treated Rats with Ethanolic Extract and

 Glibenclimide

Time(hours)	Weight of Diabetic Control (g)	Weight of rats (g) treated with glibenclamide (5 mg/kg)	Weight of rats (g) treated with ATEE (400mg/kg)
0 day	$269.8 \pm 2.7013$	$165.8 \pm 3.563$	171.4 ±4.722
7 <sup>th</sup> day	$243.8 \pm \! 3.70135^a$	167 ±6.041 <sup>a</sup>	$146.4 \pm 5.549^{a}$
14 <sup>th</sup> day	164.2 ±5.167 <sup>a</sup>	$147.4 \pm 4.9799^{a}$	$137.2\ {\pm}2.5884^{a}$

Values are expressed as Mean ±SEM significant at (p < 0.001), where (<sup>a</sup>) = (p < 0.001) vs control.

**Table 5.** Liver Enzymes (SGPT, SGPT, and ALP) Levels of Rats after

 Fourteen Days of Treatment with Ethanolic Extract and Glibenclimide

Grouping of rats	SGOT (units/l)	SGPT (units/l)	ALP (units /l)
Diabetic Control	$178 \pm 3.48$	$170 \pm 4.98$	95±2.30
Glibenclimide treated	$158 \pm 3.962^{a}$	$164.4{\pm}4.277^{ns}$	$90.4\pm\!\!6.730^a$
ATEE (400mg/kg)	$182.2\pm 3.563~^{ns}$	$171.5 \pm 3.646^{ns}$	$66.2 \pm \! 9.948^{a}$

Data are expressed as Mean  $\pm$ SEM (n=5), where (<sup>a</sup>) = (p < 0.005) and ns = non-significant as compare to diabetic control.

**Table 6.** Various Hematological Parameters of Glibenclimide andEthanolic Extract Treated Diabetic Rats after Fourteen Days of Treatment

Hematological	Units	Glibenclimide	ATEE	Diabetic
Parameters	Units	(5  mg/kg)	(400 mg/kg)	Control
Hb	g/dl	$\begin{array}{c} 13.278 \pm \\ 0.483^{\#} \end{array}$	$13.8 \pm 0.751^{\#}$	7.23±4.26
Monocytes	%	3	3.2	4

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Hematological	Units	Glibenclimide	ATEE	Diabetic
Parameters	Units	(5  mg/kg)	(400 mg/kg)	Control
Lymphocytes	%	$14.2 \pm 32.492$	$15.4 \pm 5.5497$	$11 \pm 3.42$
Neutrophils	%	$77.6\pm\!7.19$	$81.4 \pm 4.15$	90.8±2.30
Eosinophils	%	2.6	2	2.7
WBCS	lomm	10325.4	6443.6	427±4.49
WBCS	/cmm	$\pm 2.701$	$\pm 2.408$	42/±4.49
Platelets	/cmm	$770.8 \pm 7.19^{\#}$	527.6	190±2.30
Flatelets	/CIIIII	//0.8 ±/.19	$\pm 8.294^{\#}$	190±2.30
RBCS	Million/cmm	$7.486 \pm 0.091$	$7.986 \pm$	$5.49 \pm 2.78$
KDC5		7.400 ±0.091	0.5100	5.49±2.78
MCH	Pg	$18.1 \pm 0.565$	$17.42 \pm 0.661$	$15 \pm 3.90$
MCHC	%	$36.32 \pm 3.195$	$32.26 \pm \! 0.568$	25±2.79
НСТ	%	$44.14 \pm 2.197$	$43.7 \pm \!$	48±5.27
MCV	μm <sup>3</sup>	$57.28 \pm 2.755$	$54.66 \pm 3.038$	51.5±4.26

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Values are shown as (Mean  $\pm$  SEM), where (n=5), where (#) = ( $\mathbf{p} < 0.005$ ).

Table 7. Phytochemical Constituents of Ethanolic Extract

Phytochemical Constituents	Presence/Absence of Constituents
Alkaloids	+
Terpenes	+
Flavonoids	+
Sugars and phenols	+
Saponins	_

**Key:** (+) = Presence of corresponding phytochemical compound. (-) = Absence of corresponding phytochemical compound.

#### 4. DISCUSSION

The current study investigated the antidiabetic activity of *Asphodelus tenuifolius* in both normal and allaxon-induced diabetic rats. Both the ethanolic and aqueous extracts of *Asphodelus tenuifolius* notably reduced the blood glucose level in both normal and diabetic rats. The ethanolic extract in 400mg/kg dose produced more significant results as compared to the aqueous extract which shows that the active principle responsible for antidiabetic activity is more extractable in ethanolic extract. These results are in accordance with the previous investigations. Several plants such as *Thymus serpylum* [12], *Teucrium stocksianum* [3], and *Vinica rosea* [13]

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have been found to be effective in reducing the level of blood glucose in experimental animals. Alloxan is a commonly used diabetogenic chemical acting as  $\beta$ -cell cytotoxin, causing cell necrosis with subsequent development of DM [14, 15]. ROS reportedly mediates this  $\beta$ -cell cytotoxicity by increasing the calcium cytosolic level which ultimately destroys the  $\beta$ -cell. All these processes result into reduced insulin secretion with subsequent increased blood glucose level [16].

It has been documented that Asphodelus tenuifolius demonstrates antioxidant properties. It is well documented that plants with antioxidant activities possess antidiabetic potential [5, 8]. Hence, it is proposed that the phytochemical elements within the ethanolic extract of Asphodelus tenuifolius showcase a protective impact on β-cells against the oxidative damage induced by an elevated glucose concentration. These findings agree with previous investigations. Earlier research indicated that specific phytochemical compounds, such as flavonoids, found in antioxidant plants serve a protective role against reactive oxygen species (ROS). These compounds block the cytotoxic effect of ROS, particularly on the vital organs of the body, such as pancreas [12, 17]. Further, antioxidants have been reported to improve the effects of insulin on the transport of glucose into skeletal muscles [18]. The phytochemical compounds of test extract might protect the  $\beta$ -cells from the lethal effects of ROS and other oxidant species and may enhance the effect of insulin on improving the transportation of glucose into the muscles. Still, further histological and cell culture studies are required to investigate this effect of the tested plant extracts. There is another possibility that the test extracts may have the properties to stimulate the B-cells for the release of insulin, the effect produced by sulphonylureas [19, 20].

The effects of ethanolic extract were also studied on hematological parameters and liver enzyme (LFTs) levels of alloxan-induced diabetic rats for a period of fourteen days. Diabetes badly affects hematology, leading to decreased hemoglobin (Hb) level in alloxan-induced diabetic rats. The level of Hb and platelet count increased in the rats treated with extracts, as compared to diabetic control. Previously, it was reported that several plants have significant effects on the hematology of the diabetic treated rats [9]. Furthermore, the level of SGOT and SGPT in treated rats were not altered with the treatment of the tested extracts. However, it was found that ALP level significantly decreased as compared to the diabetic control group.

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Phytochemical testing of plant extracts was also carried out which revealed the presence of tannins, alkaloids, flavonoids, and terpenoids in the ethanolic extract. These might be active principles for the antidiabetic activity of Asphodelus tenuifolius. Phytochemical findings of the current study revealed the presence of alkaloids, tannins, saponins, flavonoids, and phenols. These findings are in agreement with the reported literature [21, 22]. In another study, phytochemical analysis confirmed that different types of bioactive compounds are present in the stem bark of this plant. Ethanolic, extract contains high amount of these constituents. Previous investigations proved the antioxidant, hepatoprotective, anti-inflammatory, hypoglycemic, and antinociceptive action of phenolic substances [23, 24]. Its seeds have the potential to treat atherosclerosis, diabetes, and hypertension. Traditionally, this plant has proved to be effective against inflammatory, digestive, and circulatory problems including hemorrhoids and rheumatoid arthritis [25]. It has been known to cure insulin resistance, dyslipidemia, aortic endothelial dysfunction, and oxidative stress [26].

### 4.1. Conclusion

Asphodelus tenuifolius demonstrated efficacy in reducing the blood glucose level in alloxan-induced diabetic rats, albeit with a lesser effect in preventing weight loss, compared with that of the standard drug. In the future, there is a need to design different bioassay-guided studies to explore the elaborative structure and pharmacological characteristics of various phytochemicals of this plant which may provide a new insight into drug development. Additional research is required to isolate active components of Asphodelus tenuifolius responsible for its antidiabetic properties and to clarify their exact mechanism of action (MOA).

### REFERENCES

- 1. Sikarwar MS, Patil M. Antidiabetic activity of Pongamia pinnata leaf extracts in alloxan-induced diabetic rats. *Int J Ayurveda Res.* 2010;1(4):199–204. <u>https://doi.org/10.4103/0974-7788.76780</u>
- Patel D, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*. 2012;2(4):320–330. <u>https://doi.org/10.1016/S2221-1691(12)60032-X</u>
- 3. Rashid M, Bashir S, Mushtaq MN, et al. Comparative hypoglycemic activity of different extracts of Teucrium stocksianum in diabetic

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rabbits. *Bangladesh J Pharmacol*. 2013;8(2):186–193. https://doi.org/10.3329/bjp.v8i2.14519

- Majeed Y, Shaukat MB, Abbasi KY, Ahmad MA. Indigenous plants of Pakistan for the treatment of diabetes: a review. *Agrobiol Rec.* 2021;4:44–63. <u>https://doi.org/10.47278/journal.abr/2020.028</u>
- Aslam N, Janbaz KH, Jabeen Q. Hypotensive and diuretic activities of aqueous-ethanol extract of Asphodelus tenuifolius. *Bangla J Pharmacol.* 2017;11(4):830–837. https://doi.org/10.3329/bjp.v11i4.27131
- 6. Quattrocchi U. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology (5 Volume Set): CRC press; 2012.
- 7. Qureshi R, Bhatti GR, Memon RA. Ethnomedicinal uses of herbs from northern part of Nara desert, Pakistan. *Pak J Bot*. 2010;42(2):839–851.
- Kalim MD, Bhattacharyya D, Banerjee A, Chattopadhyay S. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. *BMC Complement Altern Med*. 2010;10(1):e77. <u>https://doi.org/10.1186/1472-6882-10-77</u>
- 9. Pari L, Satheesh MA. Antidiabetic activity of boerhaavia diffusa L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol. 2004;91(1):109–113. https://doi.org/10.1016/j.jep.2003.12.013
- Jarald EE, Joshi SB, Jain DC, Edwin S. Biochemical evaluation of the hypoglycemic effects of extract and fraction of Cassia fistula Linn. in alloxan-induced diabetic rats. *Indian J Pharm Sci.* 2013;75(4):e427. <u>https://doi.org/10.4103%2F0250-474X.119823</u>
- 11. Alamgeer, Khan AQ, Ahmad T, et al. Phytochemical analysis and cardiotonic activity of methanolic extract of ranunculus muricatus linn. In isolated rabbit heart. *Acta Pol Pharm*. 2016;73(4):949–954.
- 12. Alamgeer, Mushtaq MN, Rashid M, et al. Evaluation of hypoglycemic activity of Thymus serpyllum Linn in glucose treated mice. *Int J Basic Med Sci Pharm.* 2014;3(2):33–36.

School of Pharmacy

- Ahmed MF, Kazim SM, Ghori SS, et al. Antidiabetic activity of Vinca rosea extracts in alloxan-induced diabetic rats. *Int J Endocrinol*. 2010;2010:e841090. <u>https://doi.org/10.1155/2010/841090</u>
- 14. Jorns A, Munday R, Tiedge M, Lenzen S. Comparative toxicity of alloxan, N-alkylalloxans and ninhydrin to isolated pancreatic islets in vitro. *J Endocrinol*. 1997;155:283–294.
- Alamgeer MR, Mushtaq M, Malik M, et al. Pharmacological evaluation of antidiabetic effect of ethyl acetate extract of Teucrium stocksianum Boiss in alloxan-induced diabetic rabbits. *J Anim Plant Sci.* 2013;23(2):436–439.
- 16. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res.* 2001;50(6):537–546.
- 17. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in β-cells: Type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*. 2003;52(3):581–587. https://doi.org/10.2337/diabetes.52.3.581
- 18. Peth JA, Kinnick TR, Youngblood EB, Tritschler HJ, Henriksen EJ. Effects of a unique conjugate of α-lipoic acid and γ-linolenic acid on insulin action in obese Zucker rats. Am J Physiol-Regul Integr Comp Physiol. 2000;278(2):R453–R459. https://doi.org/10.1152/ajpregu.2000.278.2.R453
- 19. Antia BS, Okokon JE, Umoh EE, Udobang JA. Antidiabetic activity of ethanolic leaf extract of Panicum maximum. *Int J Drug Dev Res.* 2010;2(3):488–492.
- 20. Rao BK, Kesavulu M, Giri R, Rao CA. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. fruit powder in alloxan-diabetic rats. *J Ethnopharmacol*. 1999;67(1):103–109. <u>https://doi.org/10.1016/S0378-8741(99)00004-5</u>
- 21. Eddine LS, Segni L, Ridha OM. In vitro assays of the antibacterial and antioxidant properties of extracts from *Asphodelus tenuifolius* Cav. and its main constituents: a comparative study. *Int J Pharm Clin Res.* 2015;7(2):119–125.

124—**(**PR-

- 22. Safder M, Riaz N, Imran M, Nawaz H, Malik A, Jabbar A. Phytochemical studies on Asphodelus tenuifolius. *J Chem Soci Pak*. 2009;31(1):122–125.
- Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention: progress, potential and promise (review). *Int J Oncol.* 2007;30(1):233– 245. <u>https://doi.org/10.3892/ijo.30.1.233</u>
- 24. Zhao B, Hall CA. Composition and antioxidant activity of raisin extracts obtained from various solvents. *Food Chem.* 2008;108(2):511–518. <u>https://doi.org/10.1016/j.foodchem.2007.11.003</u>
- Ahmed N, Mahmood A, Tahir S, et al. Ethnomedicinal knowledge and relative importance of indigenous medicinal plants of Cholistan desert, Punjab Province, Pakistan. J Ethnopharmacol. 2014;155(2):1263– 1275. <u>https://doi.org/10.1016/j.jep.2014.07.007</u>
- 26. Younis W, Schini-Kerth V, Junior AG, Nocchi SR, Silva DB, Roberts RE. Endothelium-independent vasorelaxant effect of *Asphodelus tenuifolius* Cav. via inhibition of myosin light chain kinase activity in the porcine coronary artery. *J Ethnopharmacol.* 2021;269:e113693. <u>https://doi.org/10.1016/j.jep.2020.113693</u>



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# **Review Report for Editor/Editorial Board**

## Author & Manuscript Details (For Editorial Office Only)

Manuscript Title	<b>Evaluation of Anti Diabetic Activity of Asphodelus</b> <b>Tenuifolius in Normal and Alloxan Induced Diabetic Rats</b>
Manuscript ID	5506
Date sent for review	09/07/2023
Date by which the form	24/07/2023
should be returned	

#### **Reviewer Details**

Reviewer Name	Dr. Muhammad Zaman
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Signature of Reviewer	

(Note: The reviewers identities remain anonymous to author(s)

#### **EVALUATION**

#### Section I

Introduction	Appropriate	
Literature Review	Up to the mark	
Methodology	Sufficient	
<b>Results and Discussion</b>	Up to the Mark	
Conclusion	Up to the Mark	

#### Section II

Strengths	Novelty	
Weaknesses	English can be improved	
Bibliography/	Up to Date	
References		
Suggestions/	Discussion can be further elaborated	
Recommendations		

Evaluation (Please evaluate the manuscript by grade 1-5)		
Items	Grade	
Novelty/ Contribution to existing knowledge	4	
Organization and Readability	5	
Soundness of Methodology	5	
Evidence supports Conclusion	4	
Adequacy of Literature Review	5	



#### Rating: 5 – Excellent, 4 – Good, 3 – Average, 2 – Below Average, 1 - Reject

#### **REVIEWER DECISION**

**Recommendation to Editor (Please mark "x" for appropriate option)** 

(X) Excellent, accept the submission

() Good, accept the submission with Minor revisions required

() Resubmit for review, Major revisions required

() Reject/Decline the submission

Please return the form to <u>cpr@umt.edu.pk</u>



# **Review Report for Editor/Editorial Board**

## Author & Manuscript Details (For Editorial Office Only)

Manuscript Title	<b>Evaluation of Anti Diabetic Activity of Asphodelus</b> <b>Tenuifolius in Normal and Alloxan Induced Diabetic Rats</b>
Manuscript ID	5506
Date sent for review	09/07/2023
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should be returned	

#### **Reviewer Details**

Reviewer Name	Dr. Daulat Haleem Khan	
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Signature of Reviewer	halamithan	

(Note: The reviewers identities remain anonymous to author(s)

### **EVALUATION**

#### Section I

Introduction	Introduction is well written according to the desired aspects of	
	the present study.	
Literature Review	Literature review is up to the mark well versed and explained.	
Methodology	Methodology is good according to the need of the present study.	
<b>Results and Discussion</b>	Results are well elaborated and discussed along with the	
	previous correlation.	
Conclusion	The conclusion of the study is explained excellent.	

#### Section II

Strengths	The project is novel, well organized and explained	
Weaknesses	No weakness is seen in the project except one thing, the first	
	letter of the drug name glibenclamide should be capital	
	throughout the text. i.e. Glibenclamide	
Bibliography/	References are good enough.	
References		
Suggestions/	I strongly recommend this manuscript for the publication without	
Recommendations	any further revisions	

Evaluation (Please evaluate the manuscript by grade 1-5)	
Items	Grade



Novelty/ Contribution to existing knowledge	5
Organization and Readability	5
Soundness of Methodology	5
Evidence supports Conclusion	5
Adequacy of Literature Review	5

Rating: 5 – Excellent, 4 – Good, 3 – Average, 2 – Below Average, 1 - Reject

### **REVIEWER DECISION**

#### **Recommendation to Editor (Please mark "x" for appropriate option)**

(x ) Excellent, accept the submission

- () Good, accept the submission with Minor revisions required
- () Resubmit for review, Major revisions required
- () Reject/Decline the submission

Please return the form to <u>cpr@umt.edu.pk</u>