Research Article

Assessment of Antidiabetic Potential and Phytochemical Profiling of *Viscum album*, a Traditional Antidiabetic Plant

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Received 25 May 2022; Accepted 29 June 2022; Published 4 August 2022

Academic Editor: Bilal Sadiq

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The traditional antidiabetic plant *Viscum album* (*V. album*) was collected from the oak plant in the wild at Maidan, Khyber District, of Pakistan, for assessment of its antidiabetic potential and phytochemical profile. Lowering of blood glucose level, antioxidant effect, lipids profile, liver function marker enzymes, and kidney function markers were evaluated in extract and glibenclamide treated groups, in normal as well as in diabetic control groups. An elevated level of blood glucose level, lipids (Cholesterol, TG, LDL), liver function marker enzymes (ALT, AST, ALP), and kidney function markers (bilirubin, creatinine, urea) were observed in alloxan-induced diabetic rats; however, a HDL level was decreased. Administration of *V. album* hydroalcoholic extract for 28 days renovated significantly (*p* < 0.05) all the above biochemical parameters. The antioxidant enzymes SOD, CAT, and GPx were also considerably restored. In vitro, antioxidant assay indicated that the extract of 2,000 µg/mL scavenges free radicals of DPPH 68.4%, ABTS 69.5%, and H2O2 50.6%. The extracts revealed the presence of saponins, flavonoids, alkaloids, terpene, tannins, phenols, protein, amino acids, sterols, and glycosides. The extract has shown phenolic contents 421 ± 4.8 mg GAE/100 g d. w and flavonoids content 127 ± 1.4 mg equiv. Rut/100 g d. w. The findings of this research recommend *V. album* could be a potent source of natural antidiabetic constituents.

1. Introduction

Diabetes is an abnormal health condition characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Diabetes has been found to cause a number of long-term health complications, including nephropathy, neuropathy, and cerebrovascular and cardiovascular diseases [1, 2]. The global increase in diabetes cases at an alarming rate is extraordinary. A report by World Health Organization (WHO) indicates that more than 180 million people worldwide have diabetes, which is expected to double by 2030 [3]. In 2008, an estimated 347 million people globally had diabetes, and the prevalence is rising, mainly in low- and middle-income countries. Of these, it remained undiagnosed in more than 36 million people [4]. International Diabetes Federation (IDF) estimated that 451 million adults live with diabetes worldwide in 2017, projected to be 693 million by 2045 if no effective prevention methods are adopted [5]. In 2019, diabetes was the ninth leading cause of death, with an estimated 1.5 million deaths directly caused by diabetes [6].

Currently, diabetes is treated with oral hypoglycemic agents and insulin. The oral hypoglycemic agents available in...
the pharmaceutical market include biguanides, pioglitazone, glibornuride, bromocriptine, glitazones, bezafibrate, glipizide, pioglitazone, rosiglitazone, saroglitazar, and metformin. Oral drugs are limited due to their undesirable effects on health, including cutaneous, gastrointestinal problems, hematological, hypoglycemic coma, and disturbance of liver and kidney functions [7]. In addition, they are not suitable for use during pregnancy [8]. The management of diabetes without any side effects is still a challenge for the medical system. Despite the remarkable advances in health sciences and medical care, patients are increasingly demanding to use natural products with antidiabetic activity [9]. Historically, herbal remedies and formulations have been used for centuries owing to their medicinal value, which has been extensively explored as a strategy against diabetes. In developing countries, herbal medicines have gained substantial importance due to the lack of side effects [10]. Numerous medicinal plants have been reported to be effective in treating diabetes, yet further exploration into this topic is essential. Several investigators have shown that coumarins, flavonoids, terpenoids, and other secondary plant metabolites, including arginine and glutamic acid, exhibit antidiabetic properties [11].

*Viscum album* L. Wight & Arn is a member of the family Santalaceae. It is a hemiparasitic shrub that has its root sunk into the branches and trunks of other trees instead of the soil [12]. In Pakistan, the plant grows in hills on tree trunks on the stem of the oak tree. The plant in folk medicine is used to treat diabetes, stomach problems, insomnia, chronic cramps, stroke, anxiety, heart palpitation, hypertension, atherosclerosis, difficulties breathing, hot flushing in menopause, and complementary cancer therapies [13]. Herbalists in Europe, especially in Germany, used mistletoe, young twigs, and leaves to treat circulatory and respiratory system problems [14]. Various phytochemicals such as glycosides, alkaloids, viscosotoxins, phenylpropanoids, tannins, lignin, and sugars have been reported in *V. album* collected from different host plants [15]. *Viscum* species’ pharmacological effects depend on host trees [16]. Several pharmacological and ethnobotanical studies confirmed its use in the management of diabetes. Despite this, there is no evidence to support the claim that *V. album* is an antidiabetic agent or that it contains phytochemicals. Therefore, this study aimed to investigate *V. album* for antidiabetic potential and phytochemicals based on its traditional use.

### 2. Materials and Method

#### 2.1. Plant Collection and Preparation of Extract. *V. album* was collected from the wild oak tree at Maidan, Khyber District of Pakistan, in April 2019, and kept in the shade to dry. The dried shoots of plant were ground into powder. The fine powder 600 g was put into 2 L methanol 70% and placed at room temperature for three days with a regular shake-up and then filtered using Whatman No. 3 filter paper. The filtrate was evaporated through rotary apparatus (Strike202, Italy) at 40°C to get a concentrated dry extract. The dried extract, 47.2 g, was stored in a tube for use. A plant sample was collected, dried, and mounted on a herbarium. The plant was identified by botanists at the Herbarium Department of Botany University of Science and Technology Bannu, and a voucher specimen (MU-HBD-USTB-53) [17] was deposited at the same herbarium.

#### 2.2. In Vivo Study on Experimental Animals. Experimental animals, adult albino rats of Wistar strain weighing 240–270 g, were obtained from Animal’s House Agriculture University Faisalabad, Pakistan. The rats were maintained at 23 ± 2°C with a 12 h dark and light cycle [18]. The rats were provided with water and a standard pellet diet during this period. The animals were acclimatized to the local laboratory conditions for one week before launching the experiment. Fasting rats were supplied only with water and deprived of food for at least 16 h. The *in vivo* study was conducted in the Department of Biotechnology University of Science and Technology Bannu Pakistan, with permission from the Animal Ethics Committee University of Science and Technology Bannu.

##### 2.2.1. Experimental Design for In Vivo Antidiabetic Study. The experimental design for *in vivo* study was set, and the rats were grouped into five groups, with five in each group. A group of normal rats (Group I) was considered as a normal control, and it was provided with saline water. All the other four groups, that is, II, III, IV, and V of rats, were treated with diabetes through alloxan. Group II was considered diabetic control, and Group III diabetes-induced rats were treated with a standard drug, glibenclamide 10 mg/kg body weight. Group IV and Group V diabetes-induced rats were fed with 200 mg/kg and 400 mg/kg body weight of *V. album* extract, respectively. The extract, saline water, and the standard glibenclamide were administered orally using a mouth gauge.

##### 2.2.2. Induction of Diabetes. The rats were induced with diabetes after a fasting period of 16 hours through an injection of alloxan intraperitoneally at a dose of 120 mg/kg body weight in a volume of 1 ml/Kg freshly dissolved in cold 0.9% normal saline [19]. The elevated blood glucose level of rats was examined after three days. Afterward, the rats that showed fasting blood glucose levels of more than 200 mg/dl were selected for the experiment.

##### 2.2.3. Blood Glucose Test and Serum Collection. The blood glucose level of rats was examined on days 0, 7, 14, 21, and 28 by drawing blood from the tail vein. The extract and standard drug were orally administered for 28 days. The rats were fasted overnight and were dissected under anesthesia on day 29 to collect blood. A blood sample of each animal was collected by puncturing the animal’s heart. All samples were stored in plain glass bottles. Serum was isolated from blood after clotting for 2 hours and centrifuged at 3,000 rpm for 10 minutes. Serum was poured into test tubes for further analyses.
2.2.4. Determination of Serum Lipid Profile. The serum collected was analyzed for lipid profiles, including total cholesterol, HDL, LDL, and triglycerides, with the help of commercially available kits from Gesan Productions, Italy, using a chemistry analyzer (Selectra, Netherlands).

2.2.5. Determination of Liver and Kidney Function Markers. Liver and kidney function markers alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), serum urea, creatinine, and total bilirubin were determined as described by [20] with the help of commercially available kits from Gesan Productions, Italy, using a chemistry analyzer (Selectra, Netherlands).

2.2.6. Assessment of Tissue Antioxidant Enzymes. The tissue antioxidant enzymes assay was carried out with tissue homogenization in 100 mM KH₂PO₄ (10 volumes of buffer containing 1 mM EDTA (pH 7.4)). The mixture was centrifuged at 4°C for 30 min at 12,000 g. The enzymatic potential was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals [21]. A solution of DPPH 0.3 mmol/L was prepared in methanol, and 500 μL of this solution was added to different concentrations of the extracts 62.5–2,000 μg/mL. The mixture was shaken vigorously, and absorbance was measured at 517 nm after 30 minutes of incubation at room temperature in the dark. The results were compared with the standard antioxidants. The scavenging activity was calculated by the formula: % inhibition of DPPH = (Ai-At)/Ai ×100, where Ai is the absorbance of the control, and At is the absorbance of the test sample.

2.3. Free Radical Scavenging Assay

2.3.1. DPPH Free Radical Scavenging Assay. In the DPPH assay, plant extract’s percentage free radical scavenging potential was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals [21]. A solution of DPPH 0.3 mmol/L was prepared in methanol, and 500 μL of this solution was added to different concentrations of the extracts 62.5–2,000 μg/mL. The mixture was shaken vigorously, and absorbance was measured at 517 nm after 30 minutes of incubation at room temperature in the dark. The results were compared with the standard antioxidants. The scavenging activity was calculated by the formula: % inhibition of DPPH = (Ai-At)/Ai ×100, where Ai is the absorbance of the control, and At is the absorbance of the test sample.

2.3.2. ABTS Radical Cation Assay. In this assay, free radical scavenging potential was determined using ABTS 2, (2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid disodium salt (ABTS⁺) according to the protocol previously employed by [22]. The ABTS solution (7 mM), after mixing with potassium persulfate (2.5 mM) solution, was kept in the dark for 8 hours to protect it from light. To maintain a maximum absorbance level of 0.900 ±0.02) at 745 nm and 30°C, the stock solution was diluted by fifty percent in the respective solvent. The absorbance of 300 L of the extract solution prepared in the solvent 62.5–2,000 μg/mL was measured with a spectrophotometer using 3 mL of the diluted ABTS solution. The percentage scavenging potential was measured using the following formula: Percentage scavenging potential = (Absorbance of control–absorbance of sample)/ (absorbance of control) × 100.

2.3.3. Hydrogen Peroxide Scavenging Assay. Hydrogen peroxide is a necrotic agent. Plant phytochemicals slow down the necrotic potential of hydrogen peroxide. This scavenging potential of the plant can be predicted according to the hydrogen peroxide scavenging assay by [23]. In this case, 1.4 mL of 100 mM phosphate buffer pH 7.4 was mixed with 300 μL of the extracted sample 125–2,000 μg/mL and 300 μL of 43 mM hydrogen peroxide solution. The combined solution was allowed to stand for 40 minutes at room temperature, and absorbance was measured at 230 nm. The percentage hydrogen peroxide scavenging potential of plant extract was calculated as Percentage scavenging potential = absorbance of control–absorbance of sample/absorbance of control × 100. The tests were performed in triplicate, and values were presented as means ± SD.

2.4. Phytochemicals Screening. The extract was screened to find phytochemicals such as alkaloids, phenols, flavonoids, Saponins, tannins, terpenes, protein, amino acids, and glycosides 24,25.

2.4.1. Total Phenolic Contents. The total phenolic content of the extract was determined by the Folin–Ciocalteu method [26]. The extracted sample of 200 μL (1 mg/mL) was raised to 3 mL by adding distilled water. The sample was added 500 μL of Folin–Ciocalteu reagent and mixed thoroughly for 3 minutes. After that, the mixture was added to 2 mL of 20% (w/v) sodium carbonate, incubated for 60 minutes in the
dark, and observed at 650 nm in a spectrophotometer. The amount of total phenols was measured from the calibration curve of gallic acid, and the outcomes were compared.

2.4.2. Total Flavonoids Content. The total flavonoid content was determined through the aluminum chloride solution method [27]. First, the plant extract 250 µl from the stock solution (1-5 mg/ml in respective solvent) and a standard rutin samples with a range of 15–250 µg/ml were mixed with distilled water. After that, 75 µl of 5% (w/v) sodium nitrite solution was added. The solution was added 150 µl of 10% (w/v) aluminum chloride solution after 6 minutes of incubation. Incubation for 5 minutes was followed by adding 500 µl of 1M NaOH. Distilled water was added to make the final volume up to 2.5 ml. The absorbance was measured immediately at 510 nm. All samples were run in triplicate.

2.5. Toxicity Study. The acute toxicity study was designed to find any adverse effects of *V. album* extract on rats. The rats were divided into four groups, with five rats in each group. Three groups of rats were used for experimentation, and one group was kept normal. The rats were fasted overnight and only provided fresh water the day before the experiment. This was followed by oral administration (1 mL) of *V. album* extracts 1, 1.5, and 2 g/kg to each experimental group. The groups were examined for any adverse effect of the extract on animal behaviors and death for 24 hours [28].

2.6. Statistical Analysis of Data. The raw experimental data were tabulated and statistically analyzed using GraphPad Prism 8.2.1. For accuracy and confirmation, the experiments were done in triplicate. Therefore, the values were expressed as mean ± standard deviation. One-way ANOVA and Dennett’s test were used to analyze the data in vivo experiments. A statistically significant value of \( p < 0.05 \) was justified.

3. Results and Discussion

3.1. Hypoglycemic Effect of *V. album* Extract. The extract of 200 and 400 mg/kg body weight rather than diet significantly \( (p < 0.05) \) decreased blood glucose levels in hypoglycemic rats in vivo study (Table 1). This shows that *V. album* extract contains a hypoglycemic agent responsible for lowering blood glucose levels. The diabetic control group demonstrated high blood glucose levels during experimentation for 28 days.

3.2. *V. album* Extract and Serum Lipid Level. *V. album* extract 200 and 400 mg/kg body weight in a dose-dependent manner significantly reduced serum lipid levels in rats with hyperlipidemia (Table 2). In diabetic rats, LDL-cholesterol, VLDL-cholesterol, triglycerides, and total cholesterol significantly increased, while HDL-cholesterol levels decreased. In alloxan-induced diabetic rats, the LDL levels increased to 152.4 mg/dL, while in normal rats, it was lower than 38.2 mg/dL, while the diabetic rats, after administration of *V. album* extract 200 and 400 mg/kg body weight for 28 days, lowered LDL levels gradually to 70.2 and 43.8 mg/dL, respectively, and were comparable to those of glibenclamide (45.6 mg/dL). Triglycerides levels were also increased to 200.2 mg/dL in diabetic rats, which were 94.4 in normal rats, while the diabetic rats, after oral treatment with *V. album* extract 200 and 400 mg/kg body weight for 28 days, lowered levels gradually to 134.6 and 109.8 mg/dL, respectively. This effect was nearly identical to that of glibenclamide 107.2 mg/dL. However, the restorative impact of extracting 400 mg/kg body weight for 28 days on the total cholesterol level of 135.8 ± 20.0 mg/dL was similar to that of glibenclamide 135.8 ± 23 mg/dL. In diabetic rats, the HDL was 40.4 ± 3.9 mg/dL, which was enhanced to 67.8 mg/dL with the administration of *V. album* extract 400 mg/kg body weight for 28 days.

3.3. Restorative Effect of the Extract on Liver and Kidney Function. The alloxan can also damage liver and kidney tissues. The extent of liver injury was determined by estimating serum concentration of biochemical markers, alanine transaminase (ALT), serum alkaline phosphatase (ALP), and aspartate transaminase (AST) (Table 3). The liver serum markers function was enhanced by alloxan; however, it was significantly restored \( (p < 0.05) \) in standard antidiabetic drug glibenclamide and *V. album* extract 200 and 400 mg/kg body weight treated groups in comparison to that of the diabetic control group. This restorative effect of the extract on liver function was similar to that of the control group.

The extract of 200 and 400 mg/kg body weight also significantly \( (p < 0.05) \) improved the elevated level of serum kidney profile: serum bilirubin, urea, and creatinine in alloxan-induced diabetic rats (Table 4). This restorative effect of the extract on kidney function was also comparable to that of the normal control group.

3.4. *V. album* Extract and Alloxan-Induced Oxidative Stress. Alloxan-induced oxidative stress has dramatically reduced the amount of tissue antioxidant enzymes SOD, CAT, and
has completely restored SOD, CAT, and GPX profile, and manner significantly restored these enzymes. A higher dose of Glibenclamide was higher than that of *V. album* extract was determined in a dose-dependent manner. Administration of *V. album* in a dose-dependent manner significantly restored these enzymes. A higher dose has completely restored SOD, CAT, and GPX profile, and this restorative effect of *V. album* was higher than that of glibenclamide (Table 5).

### 3.5. Antioxidant Potential

The free radical scavenging potential of plants is due to various active ingredients such as beta carotene and ascorbic acid. Such compounds can tackle free radicals and reactive oxygen species. The antioxidant potential of *V. album* extract was determined using the following antioxidant assays.

#### 3.5.1. Scavenging Potential in the DPPH Method

DPPH assay is the commonly used in vitro assay in screening natural products for antioxidant activity. The extract showed maximum DPPH radical scavenging potential of about 68.4% at the concentration of 2000 μg/mL. The standard antioxidant ascorbic acid at the same concentration showed a DPPH radical scavenging potential of 87.58%. The scavenging potential of the extract was concentration-dependent (Figure 1).

#### 3.5.2. ABTS Radical Cation Assay

The ABTS solution is prepared by mixing potassium per sulfate with ABTS, prepared by mixing potassium per sulfate with ABTS, The ABTS solution is prepared by mixing potassium per sulfate with ABTS.

### Table 2: Effect of *V. album* extract on lipid level in alloxan-induced diabetic animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>TG (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>94.4 ± 1.5</td>
<td>125.4 ± 2.6</td>
<td>71.8 ± 6.3</td>
<td>38.2 ± 1.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>200.2 ± 2.5</td>
<td>230.2 ± 3.7</td>
<td>40.4 ± 3.9</td>
<td>152.4 ± 1.6</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>107.2 ± 2.9</td>
<td>135.8 ± 2.0</td>
<td>68.4 ± 3.1</td>
<td>45.6 ± 1.1</td>
</tr>
<tr>
<td>VA 200 mg/kg</td>
<td>134.6 ± 3.1</td>
<td>153.6 ± 1.9</td>
<td>62.2 ± 5.2</td>
<td>70.2 ± 1.3</td>
</tr>
<tr>
<td>VA 400 mg/kg</td>
<td>109.8 ± 2.3</td>
<td>135.8 ± 2.3</td>
<td>67.8 ± 4.14</td>
<td>43.8 ± 1.1</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD for five determinations. Means ± SD with different superscript letters (a–d) within the column indicate a significant difference (*p < 0.05*). The diabetic control group was compared with a standard drug-treated group and VA extract-treated group. VA: *V. album*.

### Table 3: Effect of *V. album* extract on serum liver markers level in alloxan-induced diabetic animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>37.64 ± 1.1</td>
<td>43.4 ± 2.06</td>
<td>56.4 ± 2.07</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>86.6 ± 1.52</td>
<td>105.2 ± 3.49</td>
<td>112.4 ± 2.88</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>40.2 ± 1.92</td>
<td>51.8 ± 2.93</td>
<td>65.2 ± 2.77</td>
</tr>
<tr>
<td>VA 200 mg/kg</td>
<td>62.8 ± 1.48</td>
<td>78.2 ± 2.74</td>
<td>74.4 ± 1.84</td>
</tr>
<tr>
<td>VA 400 mg/kg</td>
<td>41.2 ± 1.92</td>
<td>49.0 ± 2.2</td>
<td>58.6 ± 3.6</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD for five determinations. Means ± SD with different superscript letters (a–d) within the column indicate a significant difference (*p < 0.05*). The diabetic control group was compared with the standard drug-treated and VA extract-treated groups. VA: *V. album*.

### Table 4: Protective effect of *V. album* extract on kidney functions.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum urea (mg/dl)</th>
<th>Serum bilirubin(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.464 ± 0.03</td>
<td>23.8 ± 2.39</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.814 ± 0.11</td>
<td>62.4 ± 4.67</td>
<td>1.64 ± 0.09</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.612 ± 0.03</td>
<td>30.2 ± 3.19</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>VA 200 mg/kg</td>
<td>0.678 ± 0.03</td>
<td>35.6 ± 3.05</td>
<td>0.61 ± 0.07</td>
</tr>
<tr>
<td>VA 400 mg/kg</td>
<td>0.632 ± 0.03</td>
<td>31.6 ± 3.41</td>
<td>0.39 ± 0.03</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD for five determinations. Means ± SD with different superscript letters (a–d) within the column indicate a significant difference (*p < 0.05*). The diabetic control group was compared with the standard drug-treated and VA extract-treated groups. VA: *V. album*.

### Table 5: Protective effect of *V. album* extract against alloxan-induced toxicity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Superoxide dismutase (units/mg protein)</th>
<th>Catalase (units/mg protein)</th>
<th>Glutathione peroxidase (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10.62 ± 0.16</td>
<td>10.5 ± 0.15</td>
<td>5.69 ± 0.17</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.86 ± 0.07a</td>
<td>6.38 ± 0.13a</td>
<td>3.52 ± 0.15a</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10.2 ± 0.17 ab</td>
<td>8.84 ± 0.13 ab</td>
<td>5.27 ± 0.13 ab</td>
</tr>
<tr>
<td>VA 200 mg/kg</td>
<td>9.14 ± 0.09c</td>
<td>8.38 ± 0.14c</td>
<td>4.22 ± 0.23c</td>
</tr>
<tr>
<td>VA 400 mg/kg</td>
<td>9.94 ± 0.08 ad</td>
<td>8.68 ± 0.15 ad</td>
<td>5.21 ± 0.17 ad</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD for five determinations. Means ± SD with different superscript letters (a–d) within the column indicate a significant difference (*p < 0.05*). The diabetic control group was compared with the standard drug-VA extract-treated groups. VA: *V. album*. 

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**Table 2: Effect of *V. album* extract on lipid level in alloxan-induced diabetic animals.**

**Table 3: Effect of *V. album* extract on serum liver markers level in alloxan-induced diabetic animals.**

**Table 4: Protective effect of *V. album* extract on kidney functions.**

**Table 5: Protective effect of *V. album* extract against alloxan-induced toxicity.**
forming blue-colored ABTS radicals. In this assay, the extract was evaluated to determine how much ABTS cation radicals were reduced. The extract showed 69.5% antioxidant activity at concentration 2000 μg/mL. The standard ascorbic acid showed 63% antioxidant activity (Figure 2).

3.5.3. hydrogen Peroxide (H₂O₂) Scavenging Capacity. In this scavenging study, the extract forages hydrogen peroxide in a concentration-dependent manner (Figure 3). The extract at concentration 2000 μg/mL articulated 50.6% H₂O₂ radical scavenging, whereas ascorbic acid revealed 74.7% at the same concentration.

3.6. Phytochemical Analysis. The qualitative analysis of V. album extract indicated the presence of alkaloids, saponins, flavonoids, tannins, phenols, terpenes, sterols, and glycosides. The extract was also evaluated for phytochemicals such as total phenolic and flavonoids contents. The extract has shown phenolic contents 421 ± 4.8 mg GAE/100 g dry weight, and flavonoids content 127 ± 1.4 mg equiv. Rut/100 g dry weight.

3.7. Acute Toxicity Test. The acute toxicity was evaluated through in vivo experiment, and it was found that a dose of 2 g/kg body weight showed no sign of acute toxicity or behavioural change or mortality in rats within 24 h. The amount of 2 g/kg body weight is the maximum amount for investigation of acute toxicity of the crude drug. Therefore, V. album extract can be treated as safe up to 2 g/kg, and the approximate LD₅₀ value is more than 2 g/kg.

Despite recent advancements in medical sciences, no satisfactory effective therapy is still available to cure diabetes. The elevated blood glucose level has created many health complications. The synthetic drugs used in treating diabetes are not without any side effects. This indicates that a unidirectional curative strategy through the use of synthetic drugs in managing diabetes is not fruitful. Natural remedies from medicinal plants are considered to be an effective and safe alternative treatment for diabetes. The efficacy of V. album as an antidiabetic agent and its phytochemical screening was validated to validate its traditional use. In the in vivo study, several biochemical parameters and different tissue protection analyses against alloxan-induced toxicity were performed. An animal model confirmed the safety test of the hydroalcoholic extract of V. album in an acute toxicity test. The antioxidant potential of the extract was assessed through SOD, CAT, and GPX assays in the animal model and DPPH, ABTS, and H₂O₂ in vitro assays. The extract was screened for phytochemicals such as phenols, alkaloids, flavonoids, saponins, tannins, terpenes, protein, amino acids, and glycosides.

The extract’s antidiabetic potential was assessed in alloxan-induced diabetic rats. Alloxan is chemicals that destroy the pancreas’s beta cells, leading to low insulin and high blood glucose levels [29]. V. album extract 200 and 400 mg/kg body weights have significantly lowered blood glucose in 28 days, indicating its protective role against pancreatic injury in diabetic rats. The result is parallel to the investigations of [30]. They mentioned that a significant decrease in serum glucose level and increase in serum insulin
level was observed after administration of aqueous mistletoe extract in alloxan-induced hyperglycemic rabbits and rats. In another experiment, an aqueous extract dose-dependently enhanced insulin secretion in clonal β-cells [31]. A research report has also confirmed that phenolic compounds inhibit the onset of glycation, which is the main complication of diabetes [32].

In diabetes, the amount of insulin decreases, and as a result, the amount of lipid is increased in diabetic rats due to lipolysis of stored lipids. Therefore, serum lipids, cholesterol, LDL, and triglycerides are increased. Serum lipids composition during diabetes increases, which often leads to coronary problems. Oral treatment with *V. album* extracts markedly diminished cholesterol, triglyceride, LDL, and increased HDL levels in rats with hyperlipidemia. This shows that *V. album* treatment has enhanced insulin secretion or restored beta cells of the pancreas [33].

An increase in the levels of AST, ALT, and ALP in plasma of diabetic rats indicates hepatic injury due to alloxan. This is due to the necrosis of liver cells, and as a result, these enzymes are released from the cytosol of liver cells into the blood plasma. The curative effects of *V. album* extract on liver tissues are evident from current research results and concurrence with those observed by [34] in rats. Along with this, the ameliorative effect of *V. album* extract on liver tissues can be confirmed by a gradual decrease in plasma bilirubin levels in alloxan-induced diabetes rats. It is a common observation that, during diabetes, oxidative stress is created. The oxidative stress results from increased production of free radicals, which arise due to low levels of antioxidant enzymes [35]. Administration of hydroalcoholic extract for 28 days to rats with sharp hepatic injury normalized plasma levels of aminotransferase. Such a curative effect of *V. album* was also observed in clinical trials on patients with chronic hepatitis C that confirmed decreased levels of hepatocellular enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [36].

Plasma urea and creatinine are regarded as critical markers of renal function. The level of these two markers has increased in the diabetic control group. This can be related to the malfunctioning of the kidney tissues. However, the ameliorative effect of extract on the kidney tissues is evident from the restoration of plasma urea and creatinine levels. In addition, the research demonstrated a potential protective effect against diabetes-induced renal dysfunction and normalization of liver enzymes and lipid profile [37].

The increase in free radicals in alloxan-induced diabetic rats and the reversion in these levels after administration of *V. album* extract agree with the finding by [38]. Thus, the decreased level of liver antioxidant enzymes SOD, GPX, and CAT has been restored by administering *V. album* extract. The extract enhanced the serum’s antioxidant activity, which is extremely important in preventing diabetic complications [30].

The extract has been evaluated to contain saponins, flavonoids, tannins, phenols, terpenes, steroids, proteins, amino acids, and glycosides. The phenolic content was 421 ± 7.4 mg/g of Gallic acid equivalents, and flavonoids content was 127 ± 4.1 mg/g of rutin equivalents. Thus, the significant antidiabetic effect of the extracts of *V. album* correlates to the presence of the components mentioned above, which could act independently and synergistically and halt the consequences of alloxan-induced diabetic stress. Because bio-activities of the plants extract are attributed to the presences of phytochemicals like saponins, alkaloid, terpenes, flavonoid, Tannins, proteins, glycosides, phenols, etc. [39–41].

### 4. Conclusion

*V. album* extract was safe in a dose of 2000 mg/kg body weight. The extracts with an amount of 200 and 400 mg/kg body weight had significant antidiabetic effects on alloxan-induced rats. The extract showed alkaloids, saponins, flavonoids, tannins, phenols, terpenes, steroids, and glycosides; some are considered bioactive constituents in managing free radicals and diabetes. Hence, the *V. album* extract’s chemical constituents might help check diabetic consequences and may serve as an alternative to antidiabetic drugs. Therefore, an advanced study to authenticate the use of the plant as an antidiabetic is recommended.

### Data Availability

All the available data are incorporated in the article.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Acknowledgments

The authors wish to thank Princess Nourahbint Abdulrahman University Researchers Supporting Project number: PNUERP2022R33, Princess Nourahbint Abdulrahman University, Riyadh, Saudi Arabia, for the financial support.

### References


