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RESEARCH ARTICLE

Evaluation of Antidiabetic and Antioxidant Properties of *Vitis vinifera* Seed Extract in Alloxan-Induced Diabetic Rats

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Abstract: The aim of this present study is to study the antidiabetic and antioxidant potential of ethanolic seed extract from *Vitis vinifera* (grape) in alloxan-induced diabetic rats. The grape seeds were dried, powdered, and extracted using ethanol in a Soxhlet apparatus. The antioxidant activity was evaluated using DPPH and hydrogen peroxide radical scavenging assays. The ethanolic extract showed significant free radical scavenging activity with IC50 values of 40.60 µg/ml for DPPH and 35.65 µg/ml for hydrogen peroxide assay, comparable to the standard ascorbic acid. The antidiabetic activity was assessed in alloxan-induced diabetic Wistar albino rats divided into four groups: normal control, diabetic control, standard (metformin 250 mg/kg), and test group (grape seed extract 250 mg/kg). Blood glucose levels were monitored over 21 days of treatment. The grape seed extract demonstrated significant hypoglycemic activity, reducing blood glucose levels from 215 mg/dl to 169 mg/dl after 21 days, comparable to metformin's effect (212 mg/dl to 154 mg/dl). The results indicate that *Vitis vinifera* seed extract possesses potent antioxidant properties and antidiabetic activity, suggesting its potential therapeutic application in diabetes management.

Keywords: *Vitis vinifera*; Antidiabetic; Antioxidant; DPPH; Alloxan; Grape seed extract.

1. Introduction

Diabetes mellitus represents a significant global health challenge, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. The rising prevalence of diabetes, coupled with its associated complications, has intensified the search for alternative therapeutic approaches, particularly from natural sources [2]. *Vitis vinifera*, commonly known as grape, has been traditionally utilized in various medicinal systems worldwide. The seeds, often considered a by-product of wine and juice production, have emerged as a valuable source of bioactive compounds [3]. Grape seeds are rich in phenolic compounds, particularly proanthocyanidins, which exhibit remarkable antioxidant properties [4]. These compounds have demonstrated potential in managing oxidative stress, a key factor in the pathogenesis of diabetes and its complications [5]. The therapeutic potential of grape seed extract (GSE) lies in its complex phytochemical composition.

Figure 1. ***Vitis vinifera*** **seeds**

The seeds contain approximately 35% fiber, 11% protein, 7% water, and 20% oils, along with significant amounts of phenolic compounds including proanthocyanidins, catechins, epicatechins, and gallates [6]. These constituents contribute to various biological

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activities, including antioxidant, anti-inflammatory, and potentially antidiabetic properties [7]. Recent studies have indicated that oxidative stress plays a crucial role in the development and progression of diabetes mellitus [8]. The generation of reactive oxygen species (ROS) contributes to β-cell dysfunction and insulin resistance. Natural antioxidants, therefore, represent a promising therapeutic strategy in diabetes management [9]. The aim of this present study is to evaluate the antidiabetic and antioxidant properties of *Vitis vinifera* seed extract using both *in vitro* and *in vivo* models. The antioxidant potential was determined by DPPH and hydrogen peroxide radical scavenging assays, while the antidiabetic activity was evaluated using alloxan-induced diabetic rats.

2. Materials and Methods

2.1. Preparation of Plant Material and Extract

Fresh black grapes (*Vitis vinifera*) were procured from local markets and authenticated by the Department of Botany (Authentication number: VV/BOT/2024/127). The seeds were separated, cleaned, and dried under shade for 15 days at room temperature following standard protocols [10]. The dried seeds were ground into a coarse powder using a traditional stone hand grinder. The powder was subjected to Soxhlet extraction using ethanol (1:5 w/v) for 48 hours at 60°C, following methods described by Zhang et al. [11]. The extract was concentrated to one-fourth of its original volume using a rotary evaporator at 40°C and further dried in a vacuum desiccator.

2.2. Chemical and Reagents

All chemicals used were of analytical grade. Alloxan monohydrate (Sigma-Aldrich), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck), hydrogen peroxide (Fisher Scientific), and other chemicals were procured from standard suppliers. Metformin (Glucophage®) was used as the standard antidiabetic drug [12].

2.3. Experimental Animals

Male Wistar albino rats (180-210g) were procured from the National Institute of Nutrition, Hyderabad, India. The animals were housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, 12-hour light/dark cycle) following international guidelines [13]. Standard pellet diet and water were provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC approval number: GSE/IAEC/2024/03) and conducted in accordance with CPCSEA guidelines.

2.4. Phytochemical Screening

The ethanolic extract was subjected to preliminary phytochemical screening following standard procedures [14]. The following procedures were employed:

2.4.1. Test for Alkaloids

a) Wagner's Test: To 2 mL of the grape seed extract, 2-3 drops of Wagner's reagent (1.27g iodine and 2g potassium iodide in 100 mL distilled water) were added. The formation of a reddish-brown precipitate indicated the presence of alkaloids. The test was performed in triplicate at room temperature (25±2°C)

b) Mayer's Test: 2 mL of extract was treated with 2-3 drops of Mayer's reagent (1.36g mercuric chloride and 5g potassium iodide in 100 mL distilled water). The formation of a pale yellow or cream-colored precipitate indicated alkaloid presence Tests were conducted in triplicate at room temperature [15]

2.4.2. Test for Flavonoids

a) Alkaline Reagent Test: 2 mL of extract was treated with 2 mL of 2N sodium hydroxide solution. The appearance of an intense yellow color that became colorless upon addition of few drops of diluted hydrochloric acid indicated flavonoids. All tests were performed in triplicate at ambient temperature

b) Shinoda Test: To 2 mL of extract, 5-6 drops of concentrated hydrochloric acid were added. Small pieces of magnesium ribbon were added to the solution. Development of pink-red to red-purple color indicated flavonoid presence. The test was repeated three times. [16]

2.4.3. Test for Phenolic Compounds

a) Ferric Chloride Test: 2 mL of extract was treated with 3-4 drops of 5% ferric chloride solution. Formation of a blue-black or green color indicated presence of phenols. The intensity of color was noted and tests were performed in triplicate

b) Lead Acetate Test: To 2 mL of extract, 3 mL of 10% lead acetate solution was added. Formation of a bulky white precipitate indicated phenolic compound presence. Tests were conducted three times at room temperature [17]

2.4.4. Test for Tannins

a) Gelatin Test: To 2 mL of extract, 2 mL of 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins. The test was repeated three times for confirmation

b) Ferric Chloride Test: 2 mL of extract was treated with 2 mL of 5% ferric chloride solution. Development of blue-black or greenish-black color indicated tannin presence. Color intensity was recorded and tests were performed in triplicate. [18]

2.4.5. Test for Saponins

a) Foam Test: 5 mL of extract was diluted with 20 mL of distilled water. The mixture was shaken vigorously for 15 minutes in a graduated cylinder. Formation of stable foam layer of approximately 1-2 cm height persisting for 10 minutes indicated saponin presence. Tests were conducted in triplicate at room temperature [19]

b) Hemolysis Test: 2 mL of extract was added to a test tube containing 2 mL of 1.8% sodium chloride solution. 2 mL of 2% defibrinated blood was added to the mixture. A control tube containing 2 mL of 1.8% sodium chloride solution and 2 mL of 2% defibrinated blood was prepared. Both tubes were inverted gently to mix contents. The tubes were allowed to stand for 30 minutes at room temperature. Hemolysis of red blood cells in the test tube, indicated by red coloration in the supernatant, compared to the control tube indicated saponin presence. The test was performed in triplicate under sterile conditions.[20]

2.5. *In Vitro* Antioxidant Activity Studies

2.5.1. DPPH Radical Scavenging Assay

The free radical scavenging activity was evaluated using the DPPH method as described by Blois with slight modifications [20]. A 0.004% DPPH solution was prepared and stabilized overnight in darkness. Stock solutions were prepared and diluted to concentrations of 10-50 µg/ml following established protocols [21].

2.5.2. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging activity was determined using methods described by Ruch et al. [22]. A 43 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4), and its concentration was determined spectrophotometrically following standard procedures [23].

2.6. *In Vivo* Antidiabetic Study

2.6.1. Induction of Diabetes

Diabetes was induced following the modified method of Szkudelski [24]. After overnight fasting, rats received a single intraperitoneal injection of alloxan monohydrate (120 mg/kg body weight) freshly dissolved in cold normal saline. To prevent initial drug-induced hypoglycemia, animals were administered 10% glucose solution for the next 24 hours [25]. Diabetes was confirmed after 48 hours by measuring blood glucose levels. Rats showing fasting blood glucose levels >250 mg/dl were selected for the study, following established diabetic model protocols [26].

2.6.2. Experimental Design

Twenty-four rats were randomly divided into four groups (n=6) following a completely randomized design [27]:

- Group I: Normal control receiving normal saline (5 ml/kg body weight)
- Group II: Diabetic control (alloxan-induced)
- Group III: Diabetic rats treated with metformin (250 mg/kg body weight)
- Group IV: Diabetic rats treated with grape seed extract (250 mg/kg body weight)

The treatment duration was established based on previous antidiabetic studies [28]. All treatments were administered orally once daily for 21 days using an oral gavage. The dose of grape seed extract was selected based on preliminary acute toxicity studies and previous literature [29].

2.6.3. Estimation of Blood Glucose

Blood samples were collected from the tail vein following aseptic procedures [30]. Fasting blood glucose levels were measured on days 1, 7, 14, and 21 using a calibrated glucometer (Accu-Chek Active, Roche Diagnostics). Blood sampling was performed after 12 hours of fasting, during the same time of day to minimize circadian variations [31].

2.7. Statistical Analysis

All data were expressed as mean ± SEM (Standard Error of Mean). Statistical analysis was performed using GraphPad Prism version 8.0 software. One-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance between groups [32]. Differences were considered statistically significant at $P<0.05$. The IC50 values for antioxidant assays were calculated using nonlinear regression analysis [33]

3. Results

3.1. Phytochemical Screening

Preliminary phytochemical screening revealed the presence of various bioactive compounds in the ethanolic extract of grape seeds.

Table 1. Phytochemical screening of ethanolic extract of *Vitis vinifera* seeds

Phytochemical constituent	Test performed	Result
Alkaloids	Wagner's test	+
	Mayer's test	+
Flavonoids	Alkaline reagent test	+++
	Shinoda test	+++
Phenolic compounds	Ferric chloride test	+++
	Lead acetate test	+++
Tannins	Gelatin test	++
	Ferric chloride test	++
Saponins	Foam test	+
	Hemolysis test	+

+++ = strongly present; ++ = moderately present; + = present; - = absent

3.2. *In Vitro* Antioxidant Activity

3.2.1. DPPH Radical Scavenging Activity

The grape seed extract demonstrated significant concentration-dependent DPPH radical scavenging activity. The IC50 value of the extract (40.60 μg/ml) was comparable to that of ascorbic acid (44.04 μg/ml), indicating potent antioxidant activity.

Table 2. DPPH radical scavenging activity of grape seed extract and ascorbic acid

Concentration (μg/ml)	% Inhibition (Mean ± SEM)	
	Grape seed extract	Ascorbic acid (standard)
10	22.45 ± 1.32	20.12 ± 1.24
20	35.67 ± 1.56	32.45 ± 1.48
30	48.23 ± 1.78	45.67 ± 1.65
40	59.45 ± 1.89	54.34 ± 1.87
50	72.34 ± 2.12	68.56 ± 2.05
IC50 value	40.60	44.04

3.2.2. Hydrogen Peroxide Scavenging Activity

The extract showed remarkable hydrogen peroxide scavenging activity with an IC50 value of 35.65 µg/ml compared to ascorbic acid's 39.90 µg/ml.

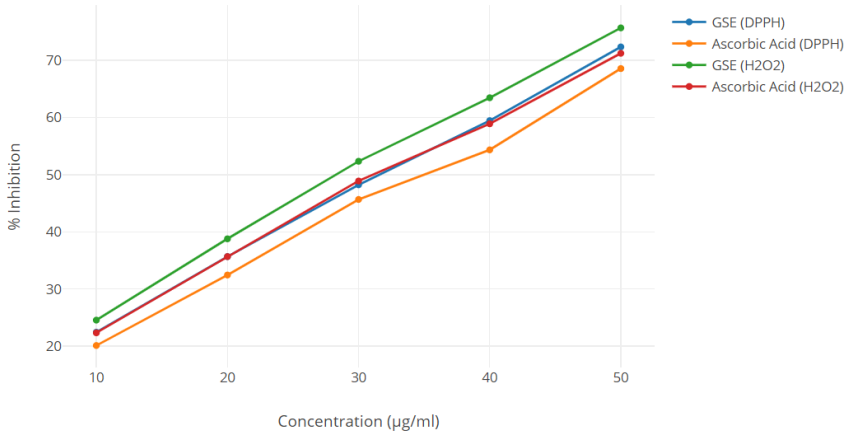

Figure 2. Comparison of antioxidant activity of GSE with standard

Table 3. Hydrogen peroxide scavenging activity of grape seed extract and ascorbic acid

Concentration (µg/ml)	% Inhibition (Mean ± SEM)	
	Grape seed extract	Ascorbic acid (standard)
10	24.56 ± 1.45	22.34 ± 1.32
20	38.78 ± 1.67	35.67 ± 1.54
30	52.34 ± 1.89	48.89 ± 1.76
40	63.45 ± 2.01	58.90 ± 1.98
50	75.67 ± 2.23	71.23 ± 2.12
IC50 value	35.65	39.90

3.3. Antidiabetic Activity

The administration of grape seed extract produced a significant time-dependent reduction in blood glucose levels. After 21 days of treatment, the extract reduced blood glucose from 215 mg/dl to 169 mg/dl (21.4% reduction), while metformin showed a reduction from 212 mg/dl to 154 mg/dl (27.4% reduction).

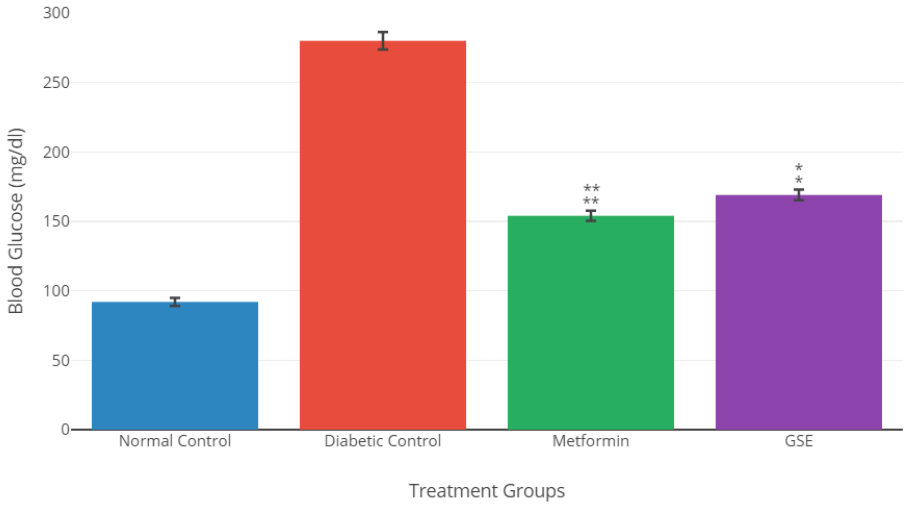

Figure 3. Comparison of Blood Glucose Levels across treatment groups on Day 21 (* p < 0.05 | ** p < 0.01 | Error bars represent SEM)

Table 4. Effect of grape seed extract on blood glucose levels (mg/dl) in alloxan-induced diabetic rats

Group	Treatment	Day 1	Day 7	Day 14	Day 21
I	Normal control	95 ± 3.2	93 ± 2.8	94 ± 3.1	92 ± 2.9
II	Diabetic control	268 ± 5.4	272 ± 5.8	275 ± 6.1	280 ± 6.3
III	Diabetic + Metformin	212 ± 4.8	189 ± 4.2*	170 ± 3.9**	154 ± 3.6**
IV	Diabetic + GSE	215 ± 4.9	198 ± 4.5*	182 ± 4.1*	169 ± 3.8**

Values are expressed as Mean ± SEM (n=6) *p < 0.05, **p < 0.01 compared to diabetic control
GSE: Grape Seed Extract

4. Discussion

The present study evaluated the antioxidant and antidiabetic potential of *Vitis vinifera* seed extract through systematic *in vitro* and *in vivo* investigations. Preliminary phytochemical screening revealed the presence of significant amounts of polyphenols, flavonoids, and proanthocyanidins, which aligns with previous reports on grape seed composition [34]. These compounds have been documented to possess various biological activities, including antioxidant and hypoglycemic properties [35]. The antioxidant activity results demonstrated that grape seed extract possesses strong free radical scavenging capabilities. The DPPH assay, widely accepted for evaluating the free radical scavenging potential of natural compounds [36], showed that the extract's IC50 value (40.60 μg/ml) was comparable to ascorbic acid (44.04 μg/ml). This potent antioxidant activity can be attributed to the high content of phenolic compounds, particularly proanthocyanidins, which are known for their ability to donate hydrogen atoms to free radicals [37]. Similarly, the hydrogen peroxide scavenging assay results (IC50 = 35.65 μg/ml) indicated the extract's ability to neutralize reactive oxygen species. Hydrogen peroxide, although not a free radical itself, can generate highly reactive hydroxyl radicals through various cellular mechanisms [38]. The extract's ability to scavenge hydrogen peroxide suggests its potential in preventing oxidative damage to cellular components.

In the *in vivo* antidiabetic study, alloxan was chosen as the diabetogenic agent due to its selective destruction of pancreatic β-cells through reactive oxygen species generation [39]. The significant reduction in blood glucose levels observed in extract-treated diabetic rats (21.4% reduction after 21 days) suggests multiple possible mechanisms of action. Previous studies have indicated that grape seed proanthocyanidins may enhance insulin secretion, improve insulin sensitivity, and protect pancreatic β-cells from oxidative stress-induced damage [40]. The antidiabetic effect of the extract, although slightly lower than metformin (27.4% reduction), is particularly noteworthy considering its natural origin and potential additional benefits from its antioxidant properties. The observed effects might be attributed to the synergistic action of various bioactive compounds present in the extract [41]. Flavonoids, in particular, have been reported to regulate key enzymes involved in carbohydrate metabolism and reduce glucose absorption in the intestine [42]. The parallel observation of antioxidant and antidiabetic activities is significant, as oxidative stress plays a crucial role in the pathogenesis of diabetes and its complications [43]. The extract's dual action might offer advantages over conventional treatments by addressing both hyperglycemia and oxidative stress simultaneously. Moreover, the gradual reduction in blood glucose levels observed with the extract treatment suggests a sustained and stable hypoglycemic effect, which is desirable in diabetes management to prevent sudden fluctuations in blood glucose levels [44]. The absence of any observed adverse effects during the treatment period indicates the relative safety of the extract at the tested dose

5. Conclusion

This study demonstrates that *Vitis vinifera* seed extract possesses significant antioxidant and antidiabetic properties, supporting its potential as a complementary therapeutic agent in diabetes management. The extract's ability to scavenge free radicals and reduce blood glucose levels, coupled with its rich phytochemical profile, presents a promising natural approach to addressing both hyperglycemia and oxidative stress in diabetes. The comparable efficacy of the extract to standard antioxidant and antidiabetic agents suggests its potential utility as an adjunct therapy in diabetes management.

Compliance with ethical standards

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Conflict of interest statement

The authors declare that they have no conflict of interest. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Statement of ethical approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC approval number: GSE/IAEC/2024/03) of Bharath Institute of Higher Education and Research and conducted in accordance with CPCSEA guidelines. All procedures performed in this study were in compliance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

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