ORIGINAL ARTICLE

Potential of Scorpion (*Scorpiops pseudomonatus*) Venom in Diabetes Therapy: A Study on α-Amylase and α-Glucosidase Inhibition

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Abstract

Background: The increasing number of diabetes mellitus patients and the complications of its treatment using chemical medicine have made it necessary to look for new treatment options that can control blood glucose levels.

Objective: To explore the possibility of using scorpion venom as an inhibitor of the essential enzymes that hydrolyze carbohydrates, glucosidase and amylase, which are important targets in diabetes treatment.

Methodology: Scorpion (*Scorpiops pseudomonatus*) venom has been evaluated for α-amylase and α-glucosidase inhibitory activities using standardized assays. Venom from selected scorpion species has been assessed for inhibitory activity using *in vitro* biochemical assays. IC_{50} values have been determined to evaluate the inhibitory potency and specificity.

Results: About 35 scorpion individual species were taken, and 0.3-0.5 milligrams of venom from every scorpion individual were collected. Significant inhibition of both α-amylase α-glucosidase and activities was shown in the results (Amylase: 9.230 ± 2.475 and Glucosidase: 2.753 ± 0.573 IC_{50} ± STD).

Conclusion: According to the results, scorpion (*Scorpiops pseudomonatus*) venom has the potential to be a unique therapeutic method for controlling blood sugar levels, as it is a promising source of natural enzyme inhibitors.

Keywords: *Diabetes mellitus, scorpion, venom, scorpiops pseudomonatus, amylase, glucosidase.*

INTRODUCTION

A group of metabolic diseases collectively known as diabetes mellitus (DM) cause mild hyperglycemia due to insufficient insulin production, insulin action, or both. Consequently, there are deficits in protein, fats, and carbohydrates [1]. There are two primary types of diabetes: type 1 and type 2 [2]. 90% of cases of diabetes worldwide are caused by type 2 diabetes mellitus, which affects 463 million people. This serious condition can be treated with both insulin and non-insulin medications. When treating non-insulin patients, several drugs are taken orally, such as glucagon-like peptide 1 receptor inhibitors, sodium-glucose Co-transporter 2 inhibitors, sulfonylureas, and metformin [3]. Type 1 and Type 2 diabetes are characterized by higher blood glucose levels brought on by either insulin resistance or a reduction in its production, respectively [4]. Currently, there are 230 million diabetics worldwide; by 2040, that figure will be expected to increase to 642 million [5-7]. Numerous risk factors, such as increased platelet aggregation, decreased fibrinolytic activity, increased production of reactive oxygen species (ROS) and nitrogen species (RNS), hyperglycemia, hypertension, dyslipidemia, atherosclerosis, and micro- and macrovascular problems, inflammatory response associated with diabetes mellitus (DM) [5-9]. Within the digestive system, α-amylase plays a critical role by facilitating the conversion of starch to glucose. Since inhibitors of α-amylase function, slowing down this process can assist in managing the amount of blood glucose after meals. Numerous natural sources' ability to inhibit α-amylase has been studied. The bioactive compounds are active in animal venom, particularly scorpion venom, have drawn attention lately. Certain peptides in scorpion venom have been found in recent studies to have α-amylase inhibitory activity. These peptides can attach to the enzyme's active site and stop it from catalyzing the starch's breakdown. One study found and reported an α-amylase inhibitor in the venom of the Buthus martensii scorpion [10]. α-glucosidase activity is required for the breakdown of carbohydrates since only monosaccharides can be easily absorbed from the intestine; all other carbohydrates must first be broken down enzymatically in the intestine. It is known that the other cellular glucosidases are required for the processing of asparagine (Asn)-linked glycoproteins and glycolipids. These enzymes are involved in several biological processes, such as immunological responses, cancer metastasis, and viral infections [11-13]. α-glucosidase inhibitors are very promising therapeutic agents for the treatment of metabolic diseases such as

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obesity, hyperglycemia, and diabetes mellitus [14]. They also proved its antiretroviral efficacy by stopping the human immunodeficiency virus from reproducing [15-18].

OBJECTIVES

The current study compared the inhibitory potency of scorpion venom with the common anti-diabetic drugs, such as acarbose.

MATERIALS AND METHODS

Scorpion's Venom Collection

Experimental work was performed at Atta-ur-Rahman School of Applied Biosciences (ASAB), (NUST) H-12 Campus, Islamabad, Pakistan in 2024. Using the procedure outlined in [19], we collected individual scorpions of the same species from the northern regions of KPK Province (Pakistan), and we used a stereomicroscope to identify them using the key "EUSCORPIUS" from www.science.marshall.edu/fet/euscorpius. Healthy adult scorpions were gathered in the northern areas of Pakistan. To keep them alive, they were given water, cockroaches to eat, and separate plastic containers. The venom was extracted from 50–55 scorpions using electrical stimulation (12–16 V, 3 ms). Centrifuging at 14,000 rpm for 10 minutes at 4 0C, the venom was dissolved in distilled water and then collected into Eppendorf tubes. Before being used, the supernatant was mixed, freeze-dried, and kept at 20°C [20] (**Figs. 1 and 2**).

Fig. (1): *Scorpiops pseudomonatus* individuals in a plastic container.

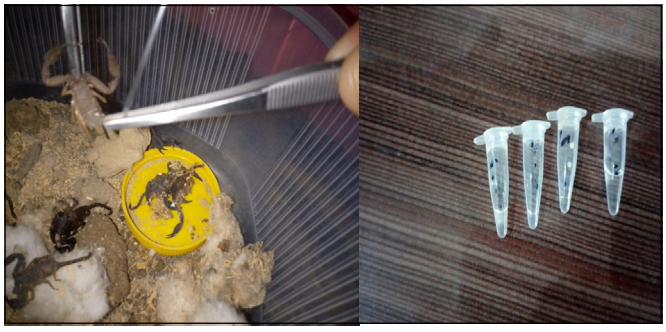

Fig. (2): Venom collection in the capillary tube by electrical stimulation of the tail.

α-Amylase Inhibition Assay

The standard protocol was slightly changed to measure the α-amylase inhibitory activity. For every experiment, 220 µl of the final reaction volume was stored in each well of the 96-well ELISA plate. 20 µl of reaction mixtures are stored in each ELISA well in the following order: DMSO (20 µl), sample (20 µl), enzyme (10 µl), phosphate buffer (50 µl), starch (20 µl), and DNS (100 µl). The substrate used in the α-amylase inhibitory experiment was starch. After adding 20 µl of DMSO to each ELISA plate well, each sample was diluted seven times. Each well was then filled with 50 µl of phosphate buffer that had a PH of 6.9. The mixture was then incubated for 20 minutes at 37°C after 20 µl of starch was added to each well. Each well received 10 µl of the α-amylase enzyme following the incubation period. Following the addition of 100 µl of DNS to each ELISA well, the wells were incubated at 37°C for 30 minutes. A 540 nm setting on an ELISA Spectrophotometer was used to determine the absorbance. The α-amylase inhibitory activity was expressed using the IC_{50} value. A plot of the percent inhibition against the log inhibitor concentration was used to calculate the IC_{50} values. These values were then based on the mean inhibitory values and derived using non-regression analysis. Acarbose was the standard. Each test was run twice [21, 22].

α-Glucosidase Inhibition Assay

Each assessment used a final reaction amount of 200 µl in each well of a 96-well ELISA plate to perform the α-glucosidase inhibition assay. Each well contained 200 µl of reaction mixtures, which were organized as follows: Phosphate buffer (130 µl), enzyme (20 µl), P-nitrophenyl-alpha-D-glucopyranoside (40 µl), and sample (10 µl). P-nitrophenyl-α-D glucopyranoside was used as the substrate in a glucosesidase inhibitory assay. The control sample in the α-glucosidase inhibition assay contained 40 µl alpha PNPG, 130 µl PBS, 10 µl DMSO, and 20 µL of enzyme, whereas the blank control merely contained 200 µl of DMSO. 20 µl of the enzyme α-glucosidase, 10 µl of DMSO, and 10 µl of each sample were diluted seven times in DMSO and added to each well of an ELISA 96-well plate. Each well received 40 µl of P-nitrophenyl-alpha-D-glucopyranoside after the phosphate buffer. After that, the mixture was incubated for 15 minutes at 37°C. We then measured absorbance at 405 nm using an ELISA spectrophotometer. The α-glucosidase inhibitory activity was expressed based on the IC_{50} value. Plotting the percent inhibition against the log inhibitor concentration allowed for the determination of the IC_{50} values. Non-regression analysis was then used to produce these

values, which were based on the mean inhibitory values. Every test was carried out twice, with acarbose acting as the standard [21, 22].

Statistical Analysis

For data analysis SPSS while for plotting figures GraphPad Prism version 9.5.1 was used to obtain possible outcomes.

RESULTS

Scorpion's Venom Collection

Scorpions of the same species were gathered from the northern areas of the KPK Province in Pakistan. These scorpions are known as *Scorpiops pseudomonatus* using a guide called the "EUSCORPIUS" key, which can be found at www.science.marshall.edu/fet/euscorpius (**Fig. 3**).

Fig. (3): Identified specie (*Scorpiops pseudomonatus*).

To collect the venom, we used electrical stimulation on the scorpion's tail (called the telson). Each scorpion provided about 0.3 to 0.5 milligrams of venom. The collected venom was stored in small tubes (Eppendorf tubes) at -20°C to keep them safe until further study is required.

In Vitro α-Amylase and α-Glucosidase Inhibitory Activity

The *in vitro* inhibition of α-amylase and α-glucosidase by the isolated scorpion's venom was investigated. The enzyme was effectively inhibited by scorpion's venom, with IC_{50} values of 9.230 ± 2.475 µM and 2.753 ± 0.573 µM, respectively. When compared to normal acarbose (IC_{50} = 16.06 ± 0.05 µM) and (IC_{50} = 16.65 ± 0.07 µM), scorpion venom showed exceptional activity.

Table 1: Tabulated data showing the Inhibitory concentration of Scorpion crude venom.

S. No	Compound	α-Amylase IC_{50} ± STD	α-Glucosidase IC_{50} ± STD
1	ScV-1	9.230 ± 2.475	2.753 ± 0.573
2	Acarbose	16.06 ± 0.05	16.65 ± 0.07

Figs. (4A&4B) illustrate the graphical depiction of scorpion venom, and Table **1** displays the IC_{50} values.

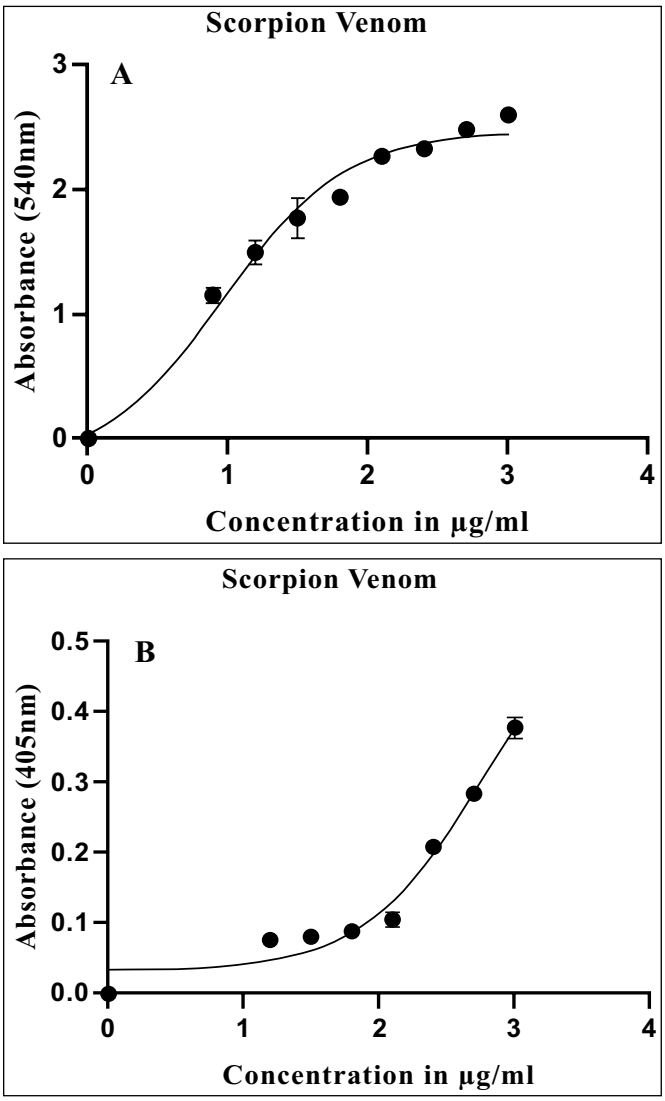

Fig. (4): (A) Dose-response curve of scorpion venom against α-amylase, **(B)** Dose-response curve of scorpion venom against α-glucosidase.

Fig. (4A) shows the dose-response curve of scorpion venom about its absorbance at 540 nm. On the x-axis the concentration of scorpion venom ranges from 0 to ~4 µg/ml and on the y-axis the absorbance increases as the venom concentration increases, indicating a concentration-dependent effect. The curve follows a sigmoidal pattern, characteristic of enzyme inhibition or saturation-type responses. At lower concentrations (< 1 µg/ml), there is a rapid increase in absorbance. At higher concentrations (> 2 µg/ml), the curve plateaus suggest that the response approaches maximum inhibition or saturation. The error bars at intermediate concentrations reflect variability in measurements. **Fig. (4B)** shows the dose-response curve of scorpion venom against a target system, with absorbance measured at 405 nm. On the X-axis scorpion venom concentration ranges from

0 to ~4 µg/ml and on the Y-axis absorbance increases progressively as the venom concentration increases. At low concentrations (0–1 µg/ml), the absorbance remains near baseline (very low change). A sharp increase in absorbance occurs between 2 to 3 µg/ml, demonstrating a concentration-dependent response. This upward trend may continue as venom concentration increases, with error bars indicating slight variability in readings.

Interpretation

The graphs show the relationship of the scorpion venom effectively inhibits the target enzyme in a concentration-dependent manner. The plateau at higher concentrations shows a point of saturation where further increases in venom concentration yield minimal changes in absorbance. These types of curves are typical for IC_{50} determination, where half-maximal inhibition occurs at a specific concentration. The steep initial slope and plateau suggest that venom is a potent inhibitor. The graph shows that the scorpion venom has a strong inhibitory effect on the enzyme system being studied with measurable responses occurring even at low concentrations. This aligns with its potent IC_{50} values reported earlier. The scorpion venom shows a delayed yet sharp increase in absorbance indicating an enzymatic inhibition or activation effect as the concentration increases. At low concentrations (< 2 µg/ml), venom has minimal impact. The significant rise beyond 2 µg/ml suggests that a threshold concentration is required to observe noticeable activity. The curve suggests enzyme inhibition kinetics, where activity remains low initially and increases steeply as venom concentration reaches effective levels. The graph reflects that scorpion venom induces measurable changes at higher concentrations, the delayed but sharp increase highlights venom potency by reinforcing its effectiveness as an active agent against the system.

Interpretation of Table 2: Statistical Comparison of Scorpion Venom *vs.* Acarbose

Table **2** matches the inhibitory activities of scorpion venom and Acarbose for α-amylase and α-glucosidase by using statistical parameters such as the difference of mean, standard error, t-statistics and p-values. The mean difference value is 6.83 which means scorpion venom shows a significantly lower IC_{50} value as compared to acarbose and shows higher inhibitory potency. The standard error of 2.476 means the error in the mean difference calculation is negligible but notable. In the table, the t-statistic value of 15.90 shows a highly significant difference between the two groups. The p-value < 0.0001 shows that the difference in IC_{50} values is statistically significant which shows scorpion venom is significantly more effective at inhibiting α-amylase compared to acarbose. In α-glucosidase inhibitory activity, the Difference of Means is 13.897 which means the IC_{50} for scorpion venom is much lower than that of acarbose, telling it is far more effective in inhibiting α-glucosidase activity. The standard error of the difference is 0.577 indicating high precision in the calculation of the mean difference. The t-statistic value is 120.11 this extremely large negative t-value indicates a substantial difference between the two groups and the p-value < 0.0001 as the extremely low p-value confirms that the difference is statistically significant. It means scorpion venom shows exceptional inhibitory activity against α-glucosidase, far exceeding that of acarbose. Both α-amylase and α-glucosidase inhibitory activities of scorpion venom are significantly higher than those of acarbose. The low p-values (< 0.0001) and high negative t-statistics confirm the robustness and reliability of the results. The smaller IC_{50} values for scorpion venom emphasize its greater potency as an enzyme inhibitor.

Statistical Significance

Performing comparison between two independent sets of data: Scorpion Venom and Acarbose for both α-Amylase and α-glucosidase inhibitory activities. Each dataset is treated as having a single observation per group, Leading to an independent test.

Interpretation of Statistical Results

p-value < 0.05: This indicates that the differences in IC_{50} values between scorpion venom and Acarbose show statistically significant inhibitory effects on both α-amylase and α-glucosidase.

t-statistic: The negative values of the t-statistic indicate that the IC_{50} values for scorpion venom are significantly lower than those for acarbose.

DISCUSSION

This study shows that scorpion venom inhibits both α-amylase and α-glucosidase, which provides strong evidence for its possible use in antidiabetic treatment. The observed significant inhibitory effects underscore the venom's capacity to regulate postprandial

Table 2: Tabulated data showing the comparison between mean, standard error t-statistics and p-value.

S. No.	Comparison	Difference of Means	Standard Error of Difference	t-statistics	p-value
1	α-amylase Inhibitory (Scorpion venom *vs.* Acarbose)	-6.83	2.476	-15.90	<0.001
2	α-glucosidase Inhibitory (Scorpion venom *vs.* Acarbose	-13.897	0.577	-120.11	<0.001

hyperglycemia, an essential component in the therapy of diabetes. The IC_{50} value of 9.230 ± 2.475 µM for the scorpion venom against α-amylase is considerably less than the IC_{50} value of 16.06 ± 0.05 µM for the common antidiabetic medication acarbose. This indicates that acarbose is not as effective as scorpion venom at inhibiting α-amylase activity. A lesser concentration of scorpion venom is needed to block 50% of the enzyme activity, as indicated by the lower IC_{50} value. This could result in a more powerful therapeutic impact at possibly lower doses. As demonstrated by earlier research, α-amylase inhibitors can successfully lower the rate of glucose absorption and carbohydrate breakdown, which helps to regulate blood glucose levels [23]. Our results align with existing investigations and imply that the active ingredients in scorpion venom may function as strong α-amylase inhibitors, providing a fresh approach to the management of diabetes. With an IC_{50} value of 2.753 ± 0.573 µM, scorpion venom's α-glucosidase inhibitory action was even more pronounced than acarbose's 16.65 ± 0.07 µM. This suggests that scorpion venom has a far stronger ability to block α-glucosidase. By delaying the breakdown of carbohydrates, inhibition of α-glucosidase lowers the rate at which glucose is absorbed and, as a result, lowers blood glucose levels after meals [24]. The efficiency of scorpion venom as an α-glucosidase inhibitor is consistent with earlier studies showing that natural substances can be superior to manufactured medications in terms of efficacy and adverse effects [25]. This research contributes to the increasing amount of data that venom-derived chemicals can be used therapeutically. The precise method by which scorpion venom inhibits α-amylase and α-glucosidase is still unclear, however, the venom probably comprises proteins or peptides that obstruct these enzymes' active sites. Numerous bioactive peptides that can target particular enzymes and receptors have been found in scorpion venom in previous studies [26]. To identify and describe these active ingredients and comprehend their exact actions, more research is required. Scorpion venom's strong inhibition of α-amylase and α-glucosidase indicates that it may be a natural source of antidiabetic substances. There is an urgent need for new medications that are safe and effective due to the growing incidence of diabetes and the shortcomings of existing therapy. With its proven ability to inhibit certain enzymes, scorpion venom presents a viable option for additional research. Furthermore, compared to synthetic medications, the natural source of scorpion venom may offer benefits in terms of biocompatibility and fewer side effects. However, to guarantee the security and effectiveness of substances derived from scorpion venom for use in humans, thorough toxicological analyses and clinical trials are necessary.

CONCLUSION

The examination indicates notable fluctuations in the effectiveness of various techniques for gathering scorpions. Rock-rolling seems to be the least successful strategy; pouring water into nests seems to be the most effective. The statistical significance indicates that these variations are caused by variations in method effectiveness rather than being the result of random chance. In comparison to the usual medication acarbose, the scorpion venom has a noticeably higher inhibitory effect against both the α-amylase and α-glucosidase enzymes. This suggests that scorpion venom has a promising potential to be an efficient antidiabetic drug, which calls for more study and development. This study highlights the potential of scorpion venom as a novel antidiabetic medication by providing solid evidence for its inhibitory actions against α-amylase and α-glucosidase. Given the strong inhibitory effects seen, scorpion venom may provide a more effective treatment than the ones now available, such as acarbose. To prepare the way for therapeutic applications, future research should concentrate on separating the venom's active ingredients, clarifying their modes of action, and carrying out thorough safety evaluations.

ETHICAL APPROVAL

Ethical approval was obtained from the Advanced Studies and Research Board Hazara University, Mansehra (REF letter No. Dir A&R/Notifications/HU/2021/193). All the procedures have been performed in accordance with Guidelines for Animal Experiments established by Hazara University, Mansehra, Pakistan.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

The data set may be acquired from the corresponding author upon a reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

SAN : Conception, design, and manuscript writing.
HV : Final approval of the manuscript to be published.
SA : Data acquisition and analysis/interpretation.
MA : Critical revision for intellectual content.
AA : Data acquisition and critical revision.

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