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# Screening of *in-vitro* Antidiabetic Potentials from the Protein Extracts of *Stomopneustes variolaris*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Diabetes mellitus (DM) is one of the serious endocrine disorders caused by insufficient production or inefficacy of the insulin and it is the immense cause of death throughout the world. However, finding for novel antidiabetic drugs from natural resources is still fascinating, because of their safe effect on DM. The marine environment provides a pool of unique and novel bioactive compounds due to its extremely diverse environmental conditions and biota. *Stomopneustes variolaris* is a marine echinoderm, belongs to family Stomopneustidae and they have toxins in their bodies as a defence mechanism which often has medicinal value. The present investigation aimed to evaluate the antidiabetic potentials of protein extracts from the spines, shell, and tissue of *Stomopneustes variolaris.* The spine, shell, tissue shows significant variation in protein content and the highest concentration of protein was found in tissue extract. The studies of haemoglobin glycation reveal that the haemoglobin glycation was increased with increasing concentration of the glucose. The highest haemoglobin glycation was reported in 20mg/ml glucose concentration at 24hr incubation

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period. The Glucose Adsorption Capacity of spine, shell and tissue extracts shows a strong positive correlation such as r = 0.9930, r = 0.9931 and r = 0.9969 corresponding to increased glucose concentration. The inhibition percentage of physiological haemoglobin glycation was depended on the glucose concentration. The protein extract of *Stomopneustes variolaris* spine (86.19 ± 0.28) showed the highest percentage of physiological haemoglobin glycation inhibition at 40mg/ml glucose concentration. The % of Glucose dialysis retardation index (GDRI) was decreased with increasing time incubation in all the tested protein extracts. The spine extract of *Stomopneustes variolaris* (37.26 ± 0.4) showed the highest percentage of GDRI at 30min incubation period. The maximum percentage of glucose uptake by yeast was observed with the spine extract (46.4±0.25) of *Stomopneustes variolaris.* The *in-vitro* α-amylase inhibitory activities of *Stomopneustes variolaris* spine, shell, and tissue were found as 51.11±0.79%, 41.81±0.31% respectively.

*Keywords: Diabetes mellitus; haemoglobin; glucose; α-amylase; protein extract; GDRI.*

## 1. INTRODUCTION

Diabetes mellitus is one of the serious metabolic disorders of glucose caused by inherited or acquired deficiency in the production of insulin or by the ineffectiveness of the insulin and its results in hyperglycemia due to abnormal metabolism of carbohydrates which leads to the elevated levels of glucose in the blood. The long-term hyperglycemic conditions of diabetes are associated with damage, dysfunction, and failure of various organs [1]. Diabetes is the rapidly increasing lethal disease to mankind all around the world [2]. The World Health Organization (WHO) listed diabetes as the fifth leading cause of death in 2015 and it estimated over 382 million people were suffered from diabetes in 2013, this number is increased to 500 million by 2030. According to the IDF data from 2019, there are 87.9 million diabetics residing in the middle-income countries of Southeast Asia. Particularly, the disease was more prevalent in urban areas and cities, with percentages of 34.3 and 49.4 respectively [3]. In India the current epidemic position of diabetes is increased rapidly with more than 62 million diabetic cases [4]. In the year 2000, India (31.7 million) has a top-ranked with the highest number of diabetic people followed by China (20.8 million) and the United States (17.7 million). Indians are more prevalent to diabetes when compared to the Europeans due to the lower body mass index (BMI) of Indians [5]. In the treatment of diabetes, the major problem with the antidiabetic medicine available in the pharmaceutical market is the continuation of diabetic associated with long-term complications and side effects. Diabetes is a severe economic burden on Indians, with regional variations, and it was revealed that the cost of drugs accounted for a substantial portion of the diabetic expenses. This might be minimised by the development of new generic drugs and health care systems [6]. Hence, searching for new antidiabetic drugs from natural resources is still attractive to researchers because they have substances that can be used as an alternative to antidiabetic medicines to reduce side effects.

The marine environment provides a pool of unique and novel bioactive substances because of its highly varied environmental conditions and diverse biota. The unavailability of sufficient and efficient medicines for the increasing incidence of various diseases has directed to the finding of new bioactive materials from marine sources. Marine organisms show an extensive range of bioactive compounds and the isolation of natural products from marine organisms has been exceeded to 18000 [7]. Sea urchin is a member of echinoidea class in the Echinodermata phylum and they have been considered as a potential source of bioactive substances with pharmaceutical applications [8]. The structure and quantity of bioactive compounds can vary among diverse sea urchin types possibly due to their different diet habits and physiological stages like variation in the reproduction phase [9]. Some sea urchins can synthesise toxins for their defence purpose, which often have a biomedical significance [10]. Basing on the bioactive potency, the present research has been carried out on *Stomopneustes variolaris* for the evaluation of their pharmacological activities against diabetes.

*Stomopneustes variolaris* is a tropical, rock-boring echinoid found at the intertidal zone and distributed from southern and northern poles to the equator [11]. *S. variolaris* is a commonly found sea urchin in India which is distributed along the coast of Visakhapatnam to Kanyakumari. The structure of *S. variolaris* has a hard-calcareous shell known as a test and it is

covered with spines for locomotion and defence. The mouth is positioned on its ventral side containing five calcareous plates called "Aristotle's Lantern". This mouth opens into the digestive tract that is connected to the anus which is located on the top of the test. The present investigation was designed to screen the antidiabetic properties of protein extracts from the spines, shell, and tissue of *Stomopneustes variolaris.*

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Young and healthy *Stomopneustes variolaris* were collected from Tenneti Park (Long: 83°20' 59.94'' E; Lat: 17° 44' 48.36'' N) and have been located on the North East coast of Andhra Pradesh, connected to the Bay of Bengal, Visakhapatnam, India. The collected organisms were aseptically transported to the laboratory and cleaned with distilled water. Then the samples were dissected and separation of the spines, shell, and tissue was done for the research.

### 2.2 Extraction and Estimation of Total Protein

Ferreira et al., [12] method was adopted to extract the proteins. 5 gm of *Stomopneustes variolaris* spine, shell, and tissue samples were homogenized separately in 50 mM sodium phosphate buffer containing 10% insoluble PVP and incubated at 4°C for overnight. Then the homogenates were centrifuged at 14000 rpm for 20 min at 4°C. The supernatant was separated and maintained at -20°C for further investigation. The total protein content in the spine, shell, and tissue extracts of *Stomopneustes variolaris* was quantified by adopting the Lowry et al., [13] method. The protein content was expressed in mg per gm fresh weight.

### 2.3 Evaluation of Haemoglobin Glycation

#### 2.3.1 Preparation of haemoglobin

The blood was collected from a healthy human volunteer in a vial containing an anticoagulant and the collected blood was washed three times with 0.14M NaCl. Two volumes of 0.01M phosphate buffer (pH 7.4) and 0.5 ml $CCl_4$ was added to the blood suspension to lyse the RBC. At room temperature the haemolysate was centrifuged for 15 min at 2300 rpm. After centrifugation, the haemoglobin rich upper layer was isolated and kept in a refrigerator until use [14].

#### 2.3.2 Estimation of haemoglobin glycation

Three distinct concentrations (2, 10, and 20 mg/ml) of glucose solution (pH 7.5) was used to estimate haemoglobin glycation. 1ml of haemoglobin was taken in a series of three test tubes each containing 1ml of 2, 10, and 20 mg/ml glucose solution. The haemoglobin fraction without the glucose was used as a blank. The test tubes were incubated for different time intervals such as 0, 24, 48, & 72 hrs and the degree of glycation was quantified by estimating the liberated hydroxymethylfurfural [14].

### 2.4 Determination of Extract Glucose Adsorption Capacity

Five distinct concentrations (5, 10, 20, 50, and 100 mg/dl) of glucose solution was used to estimate Glucose adsorption capacity of extract. 0.25ml of *Stomopneustes variolaris* spine, shell, and tissue protein extracts were separately taken in a series of five test tubes each containing 25ml of 5, 10, 20, 50, and 100 mg/dl glucose solution. The test tubes were incubated for 6hrs at 37°C in a shaker water bath. Then, the tubes were centrifuged for 20 min at 4800 rpm and the glucose content was measured in the supernatant [15]. The amount of glucose bound to the extract was measured by the below formula.

$$Glucose\ bound = 100 - \frac{G1 - G6}{Sample\ Weight} \times Solution\ Volume$$

G1 is the amount of glucose in original solution.
G6 is the amount of glucose after 6 hr incubation.

### 2.5 Effect of Extract on Physiological Haemoglobin Glycation

A series of increasing glucose concentrations (1-40mg/ml) were used to study the inhibition percentage of physiological haemoglobin glycation with the protein extracts of *Stomopneustes variolaris* spine, shell, and tissue. 1ml of haemoglobin was taken in a series of five test tubes each containing 1 ml of 1, 10, 20, 30 and 40mg/ml glucose solution. To the above test tubes, 5µl of gentamycin and 30µg/ml of protein extracts were added. The positive

control was maintained by adding 30µg/ml gallic acid instead of protein extracts and the contents were gently mixed and incubated at room temperature for 72 hrs in dark. After incubation, the free haemoglobin was estimated by spectrophotometrically at 443nm to measure the degree of inhibition percentage haemoglobin glycation [14].

## 2.6 Effect of Extracts on in-vitro Glucose Diffusion

The in-vitro glucose diffusion study was performed with increasing incubation times. A mixture of 25ml glucose solution (20mM) and 1% protein extracts of *Stomopneustes variolaris* spine, shell, and tissue were separately packed in a dialysis bag. The bags were dialysed against 200ml distilled water at $37^0$C and the amount of glucose in the dialysate was evaluated at 30-, 60-, 120, and 180-min incubation times. Control was maintained without protein extract [16].

$$\%\, GDRI \; = \; 100 - \frac{Glucose\ content\ in\ the\ Test\ (mg/dl)}{Glucose\ content\ in\ the\ control\ (mg/dl)} \times 100$$

## 2.7 Effect of Extracts on Glucose Uptake in Yeast Cells

Commercial baker's yeast was used to study the effect of protein extracts on glucose uptake. 5gms of yeast was dissolved in distilled water and allowed to the repeated centrifugation at 3000 rpm for 5min till getting the clear supernatant. From the clear supernatant, 10% yeast suspension was prepared with distilled water. 1ml of protein extracts from *Stomopneustes variolaris* spine, shell, and tissue were taken in separate test tubes each contains 1ml of 100mM glucose solution and the tubes were incubated at $37^0$C for 10min. Control was kept without the addition of protein extracts. To the above tubes, 100µl of 10% yeast suspension was added and they were permitted to incubation for 60min at $37^0$C. After incubation, the tubes were centrifuged at 2500 rpm for 5min and the amount of glucose was quantified in the supernatant.

$$\%\, of\ glucose\ uptake = \; 100 - \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

## 2.8 α-Amylase Inhibitory Assay

The α-amylase inhibitory assay for protein extracts of *Stomopneustes variolaris* spine, shell and tissue were evaluated according to the methodology of Malik and Singh et al., [17] with minor alterations. 1ml of protein extracts and 1ml of 0.5 mg/ml α-amylase solution were taken in test tubes and the tubes were allowed incubate at room temperature for 10min. To the above mixture, 1ml of 1% starch was added and again incubated the mixture at room temperature for 10min. After incubation, 2ml of dinitrosalicylic acid was added in all the test tubes to terminate the reaction and the tubes subsequently kept in a water bath for 5min at $100^0$C. Once the temperature of the reaction mixture reached the room temperature, the mixture was diluted by adding 10ml distilled water and absorbance was measured at 540nm. Control was maintained by adding the buffer instead of protein extract and acarbose was used as standard.

$$\%\, of\ \alpha\ amylase\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

# 3. RESULTS AND DISCUSSION

## 3.1 Protein Estimation

Screening of protein quality and quantity gives an idea about the habitat and medicinal importance of an organism. Different marine organisms that live in different habitats show a significant variation in the quantity of proteins. Mayne and Robinson [18] reported the positive relationship between total body protein and feeding habit of an organism. The protein content in the spine, shell, and tissue of *Stomopneustes variolaris* shows a large variation. The protein concentration in the tissue of *Stomopneustes variolaris* was found as 11154±170 µg/gmFW. Whereas, spine and shell extracts have 764±7.8µg/gmFW and 1565.56±7.37 µg/gmFW of protein respectively. Among all the three extracts of *Stomopneustes variolaris* tissue shows dominant protein content.

The information of organisms' bioactive potential is very important since the therapeutic value is reflected by its biomolecular composition. Protein is a chief biochemical component which plays a vital role in determining the structure and quality of an organism [19]. Protein is one of the dominant biochemical constituents in *S. variolaris* and it was high in tissue than exoskeleton. Mol et al., [20] carried out similar research in *P. lividus* and the protein content was observed as 12.03 ± 1.26%. Shikov et al., [21] and Bragadeeswaran et al., [22] reported the protein content in *S.*

*droebachiensis* and *T. toreumaticus* as 39.7% and 2.70 mg/ml correspondingly. Kato and Schroeter, [23] reported that *S. franciscanus* and *S. purpuratus* show variation in protein percentage which ranges from 7.7 to 9.6 and 9.5 to 12.3 respectively. The present findings are correlated with some earlier works and also some differences are reported. These differences are associated to the omnivorous feeding habit and adequate food present in the surrounding.

## 3.2 Estimation of Haemoglobin Glycation

The evaluation of haemoglobin glycation has a crucial role in antidiabetic studies. The maximum haemoglobin glycation was observed at 24hr incubation period in 20mg/ml concentrated glucose solution. At 24 hr incubation period the haemoglobin glycation was reported as 0.333±0.005, 0.145±0.004 and 0.546±0.005 with respect to the 2, 10 and 20 mg/ml glucose concentrations (Fig. 1). At 48 hr incubation period the haemoglobin glycation was reported as 0.441±0.006, 0.346±0.005 and 0.277±0.005 with respect to the 2, 10 and 20 mg/ml glucose concentrations (Fig. 2). At 72 hr incubation period the haemoglobin glycation was reported as 0.143±0.005, 0.189±0.005 and 0.217±0.005 with respect to the 2, 10 and 20 mg/ml glucose concentrations (Fig. 3). Increased glucose concentration in the blood leads to glycation of haemoglobin which may induce the production of reactive oxygen species. An increasing pattern of glycation was observed on the incubation of haemoglobin with the increasing concentration of glucose (2mg, 10mg, and 20mg) over a period of 72hr.

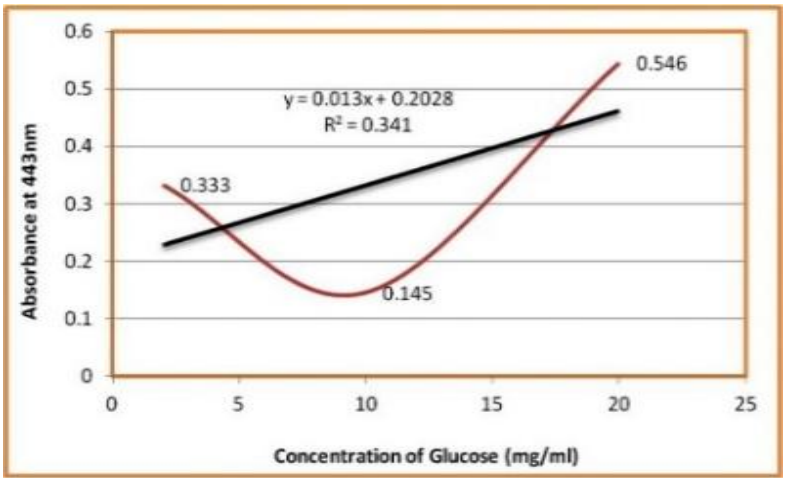


**Fig. 1. Haemoglobin glycation with three glucose concentrations at 24hr time period**

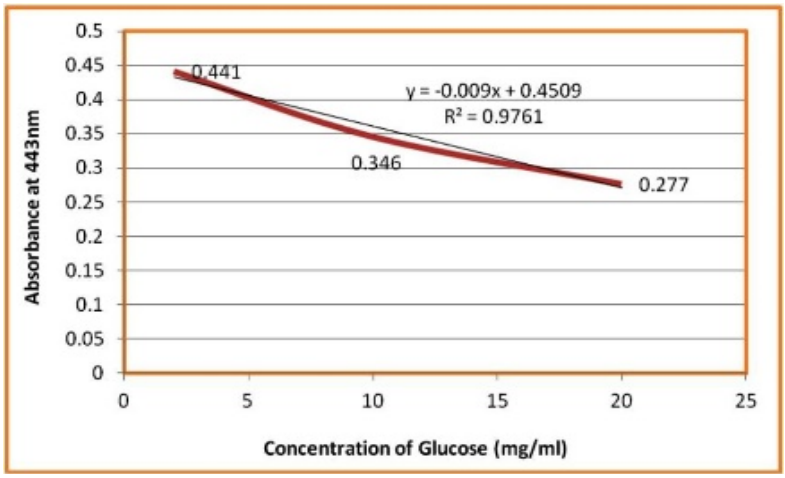


**Fig. 2. Haemoglobin glycation with three glucose concentrations at 48hr time period**

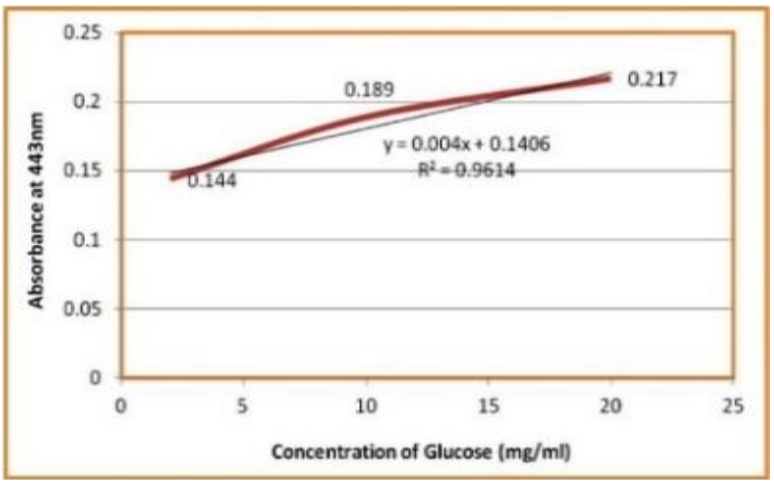


**Fig. 3. Haemoglobin glycation with three glucose concentrations at 72hr time period**

## 3.3 Glucose Adsorption Capacity

The glucose adsorption capacity of spine, shell, and tissue protein extracts from *Stomopneustes variolaris* was increased with the increasing concentration of glucose. It was observed that all the protein extracts of *Stomopneustes variolaris* showed similar Glucose Adsorption Capacity at 100mm and 5mm glucose concentration. At 10mm glucose concentration, the Glucose Adsorption Capacity of *Stomopneustes variolaris* spine, shell, and tissue protein extracts were reported as 6.436±0.09, 7.99±0.11, and 9.67±0.11 respectively. Whereas at 20 mm glucose, the Glucose Adsorption Capacity was reported as 10.143 ± 0.06, 8.804±0.02 and 16.490±0.06mg/dl for the spine, shell, and tissue protein extracts respectively. All the tested protein extracts show maximum glucose adsorption capacity was observed at 10 and 20mm glucose concentration. The IC50 values for the spine, shell, and tissue protein extracts of *Stomopneustes variolaris* were measured as 71.401, 59.664 and 140.704 mm respectively.

These results illustrate that the Glucose Adsorption Capacity was depended on the glucose concentration. The Glucose Adsorption Capacity of all the *Stomopneustes variolaris* protein extracts was significantly (<.0005) increased with increasing glucose concentration. The protein extracts of *Stomopneustes variolaris* spine, shell, and tissue show a strong positive correlation such as r = 0.9930, r = 0.9931 and r = 0.9969 correspondingly with increasing glucose concentration. The shell protein extracts show a more positive correlation of Glucose Adsorption Capacity (r = 0.9969) when compared to the Glucose Adsorption Capacity of the spine, and tissue in response to increasing glucose concentration. The results were shown in Table 1.

The present results demonstrated that the tested protein extracts possess a captivating glucose adsorption capacity. Furthermore, all the extracts show a positive correlation of glucose adsorption capacity with the glucose concentration. A similar observation was reported by Bhutkar and Bhise [24], the adsorption capacity of the extracts of *Albizzia lebbeck* and *Mucuna pruriens* show a direct relationship to the glucose concentration. Invitro and Invivo studies of Chau et al., [25] demonstrated that the density of soluble polysaccharides causes late glucose absorption by gastrointestinal cells. El Barky et al., [26] has been reported that the saponins content in the sea cucumber acts as an antidiabetic agent.

## 3.4 Effect of Extracts on Physiological Haemoglobin Glycation

The spine protein extracts of *Stomopneustes variolaris* exhibit the highest (86.19 ± 0.28) inhibition percentage of physiological haemoglobin glycation at 40mg/ml glucose concentration. Whereas, at 1mg/ml glucose concentration the inhibition percentage of physiological haemoglobin glycation by spine protein extract was reported as 78.56±0.30. The protein extracts of *Stomopneustes variolaris* shell and tissue have no activity at 1mg/ml glucose. Whereas, at 40mg/ml glucose concentration, inhibition percentages of shell and tissue extracts

were found as 26.45± 0.64 and 70.76±0.70 respectively. The $IC_{50}$ values of spine, shell and tissue protein extracts were calculated as 10.089, 50.696 and 15.382 mg/ml correspondingly for the inhibition percentage of physiological haemoglobin glycation. The results have been shown in Table 3.

These results demonstrated that the inhibition percentage of physiological haemoglobin glycation depended on the glucose concentration. Among all the protein extracts spine show a strong negative correlation (r = -0.1331) with increasing glucose concentration. Whereas, protein extract of shell (r = 0.80951) and tissue (r = 0.89937) shows positive correlation. The inhibition percentages of shell and tissue show a moderate positive correlation (r = 0.3223) and moderately negative correlation (r = -0.3767) respectively when compared to the inhibition percentages of spine extract. Furthermore, the tissue extracts show a positive correlation (r = 0.7377) when compared to the shell protein extracts. The results have been shown in Table 4.

The present results were found to be collinear with those of Karimabad et al., [27] who reported that the extracts of *Citrullus colocynthis* significantly inhibited the formation of glycated haemoglobin with increasing time under hyperglycemic conditions. In the management of diabetes proper attention has been given to the increased blood glucose concentration. Because it can bind to the haemoglobin which may result in the formation of the reactive oxygen species [28]. In vivo, the occurrence of this process contributes to the elevated plasma peroxides found in diabetics complication and may contribute to protein modification reactions perform with glucose in vitro. Thus, with the high level of glucose in the blood, erythrocytes have become important in the pathophysiologic mechanisms in diabetics as shown from several abnormal features demonstrated for red cells of diabetes mellitus patients [14]. Mathur et al., [29] stated that several natural bioactive compounds exhibit significant therapeutic potentials against diabetes and related diseases by inhibiting the protein glycation such as haemoglobin glycation.

**Table 1. Effect of glucose adsorption capacity of *Stomopneustes variolaris* spine, shell and tissue protein extracts on different glucose concentrations**

| S/N | Glucose Conc. (mm) | Glucose adsorption capacity mean ± Std (mg/dl) | | |
|---|---|---|---|---|
| | | Spine | Shell | Tissue |
| 1 | 5 | 4.491±0.08 | 4.304±0.017 | 4.721±0.051 |
| 2 | 10 | 6.436±0.09 | 7.993±0.116 | 9.669±0.111 |
| 3 | 20 | 10.143±0.06 | 8.804±0.020 | 16.490±0.063 |
| 4 | 50 | 54.166±0.92 | 57.999±0.310 | 57.694±0.53 |
| 5 | 100 | 133.118±0.75 | 133.394±0.096 | 133.650±0.56 |

**Table 2. Correlation of glucose adsorption capacity of *Stomopneustes variolaris* spine, shell and tissue protein extracts on different glucose concentrations**

| | Glucose Conc. (mm) | Tissue | Spine | Shell |
|---|---|---|---|---|
| **Glucose Conc. (mm)** | 1 | | | |
| **Tissue** | 0.993095 | 1 | | |
| **Spine** | 0.993183 | 0.999381 | 1 | |
| **Shell** | 0.996886 | 0.999116 | 0.998493 | 1 |

**Table 3. Effect of *Stomopneustes variolaris* protein extracts on inhibition physiological haemoglobin glycation with increasing glucose concentrations**

| S/N | Glucose Conc. (mg/ml) | Inhibition of physiological Hb glycation mean ± Std (%) | | |
|---|---|---|---|---|
| | | Spine | Shell | Tissue |
| 1 | 1 | 78.56±0.3 | 0 | 0 |
| 2 | 10 | 56.46±0.24 | 0 | 0 |
| 3 | 20 | 3.48±0.2 | 8.51±0.47 | 61.52±0.55 |
| 4 | 30 | 5.33±0.09 | 3.38±0.53 | 56.63±0.56 |
| 5 | 40 | 86.19±0.28 | 26.45±0.64 | 70.76±0.7 |

**Table 4. Correlation of *Stomopneustes variolaris* protein extracts on inhibition of physiological haemoglobin glycosylation with increasing glucose concentrations**

| | Glucose Conc. (mg/ml) | Spine | Shell | Tissue |
|---|---|---|---|---|
| **Glucose Conc. (mg/ml)** | 1 | | | |
| **Spine** | -0.133119929 | 1 | | |
| **Shell** | 0.809511439 | 0.322304 | 1 | |
| **Tissue** | 0.899379331 | -0.3767 | 0.737744 | 1 |

## 3.5 Effect of Extracts on in-vitro Glucose Diffusion

The spine protein extract of *Stomopneustes variolaris* (37.26 ± 0.4) showed the highest percentage of Glucose Dialysis Retardation Index (GDRI) at a 30min incubation period. At the 240min incubation period, the GDRI by spine protein extract was reported as 1.64±0.04. The GDRI percentage of shell and tissue protein extracts of *Stomopneustes variolaris* were observed as 31.62±0.52 and 35.84±0.37 respectively at a 30min incubation period. Whereas, at 240min incubation period the GDRI percentage of shell and tissue protein extracts of *Stomopneustes variolaris* were observed as 1.34±0.05 and 1.67±0.04 respectively. The results of the %GDRI have been shown in Table 5. These results suggested that the percentage of GDRI was significantly (<.0005) decreased with the increasing time incubation periods. All protein extracts from the spine, shell, and tissue of *Stomopneustes variolaris* shows strong negative correlation such as r = -0.91447, r = -0.92543, and r = -0.92773 correspondingly with increasing time incubation. The shell protein extracts show (r = 0.997522) a more positive correlation when compared to the spine extracts. Furthermore, the % GDRI of tissue shows a strong positive correlation such as (r = 0.999053) and (r = 0.996462) when compared to the % GDRI of spine and shell extracts respectively. The results have been shown in Table 6.

GDRI measures the invitro index for the detainment of glucose absorption in the gastrointestinal tract by the extract [30]. The greater GDRI value suggests a higher glucose retardation index. Bailey, [31] stated that the retardation of glucose diffusion may cause by the inhibition of sugar hydrolysing enzymes such as α-amylase with the extracts and delay the glucose liberation from the starch thereby decrease glucose absorption and finally reduce the blood glucose levels. The present results demonstrate that the inhibition of α-amylase enzyme may be one of the probable mechanisms of *Stomopneustes variolaris* protein extracts for exerting their hypoglycemic effect.

## 3.6 Effect of Extracts on Glucose Uptake in Yeast Cells

The transport mechanism of glucose through the yeast membrane has become an attractive method to evaluate the hypoglycemic effect of several bioactive compounds under in vitro conditions. In the present study, it was found that the protein extracts of *Stomopneustes variolaris* spine, shell and tissue showed 46.4±0.25, 28.41±0.125 and 40.09±0.4% respectively. Among all the protein extracts spine shows a higher percentage of glucose uptake in yeast. The results have been shown in Fig. 4. The *in vitro* assays of the present study indicated that the protein extracts of *Stomopneustes variolaris* possess good anti-diabetic activity.

These findings are supported by the findings of Pulivarthi et al., [32], who demonstrated that the methanolic leaf extract of *A. reticulata* enhances glucose absorption across the yeast cell membrane by 48.55%, indicating a substantial antidiabetic potential. The treatment of yeast cells with the *Stomopneustes variolaris* protein extracts, the glucose uptake was increased than the standard drug metformin. Illiano and Cuatrecasas, [33] reported that the sugar transportation across the membrane of yeast cell is though the facilitated diffusion which is mediated by the stereospecific membrane carriers. Teysink et al., [34] reported that the facilitated carriers transport the solutes from high concentration to low concentration. Hence, effective glucose transport is achieved, when the cell has low glucose concentration. The data obtained from the present studies reveal that the protein extract of *Stomopneustes variolaris* spine effectively enhances glucose uptake by yeast and induces the effective glucose utilization, thus regulating the glucose levels in the blood.

## 3.7 α- Amylase Inhibitory Assay

The carbohydrate digestion in the intestine is mediated by the hydrolytic activity of pancreatic α-amylase. Hence, researchers focus on the α-

amylase activity for *in-vitro* evaluation of the hypoglycemic effect of bioactive compounds. The *in-vitro* α-amylase inhibitory studies demonstrated that *Stomopneustes variolaris* spine, shell and tissue protein extract show significant anti-diabetic activity. The *in-vitro* α-amylase inhibitory activities of *Stomopneustes variolaris* spine, shell, and tissue protein extracts were observed as 51.11±0.79%, 41.81±0.31%, and 27.6±0.27% correspondingly (Fig: 5).

The current findings of α-amylase inhibition were strongly associated with the studies of Kifle et al., [35] who reported that the ethyl acetate extract of *H. abyssinica* exhibits significant α-amylase inhibitory activity with an IC50 of 52.11 µg/ml. Roux et al., [36] reported that the decreasing of postprandial hyperglycemia is due to the inhibition of α-amylase in diabetic patients. Hara and Honda [37] suggested that diabetes is effectively controlled by the inhibition of α-amylase activity in the gastrointestinal tract of humans and thereby reduces the absorption of simple sugars. Hence, diabetic patients need to maintain low levels of α-amylase to regulate their blood glucose levels.

**Table 5. Effect of *Stomopneustes variolaris* protein extracts on in-vitro glucose diffusion (%GDRI) with increasing incubation times**

| S/N | Incubation time (min) | Glucose dialysis retardation index mean ± Std (%) | | |
|---|---|---|---|---|
| | | Spine | Shell | Tissue |
| 1 | 30 | 37.26±0.4 | 31.62±0.52 | 35.84±0.37 |
| 2 | 60 | 21.05±0.2 | 19.87±0.13 | 20.72±0.12 |
| 3 | 120 | 9.27±0.12 | 7.67±0.12 | 10.53±0.14 |
| 4 | 180 | 3.67±0.07 | 2.98±0.04 | 4.02±0.08 |
| 5 | 240 | 1.64±0.04 | 1.34±0.05 | 1.67±0.04 |

**Table 6. Correlation of *Stomopneustes variolaris* protein extracts on *in-vitro* glucose diffusion (%GDRI) with increasing incubation time**

| | Incubation time (min) | Spine | Shell | Tissue |
|---|---|---|---|---|
| **Incubation Time (min)** | 1 | | | |
| **Spine** | -0.91447 | 1 | | |
| **Shell** | -0.92543 | 0.997552 | 1 | |
| **Tissue** | -0.92773 | 0.999053 | 0.996462 | 1 |

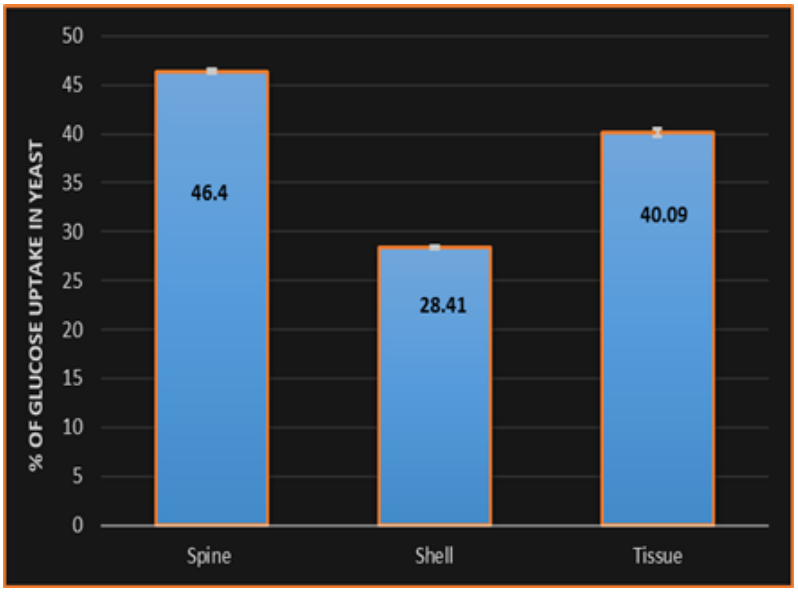


**Fig. 4. Percentage of glucose uptake in yeast cells by protein extracts of *S. variolaris* spine, shell and tissue**

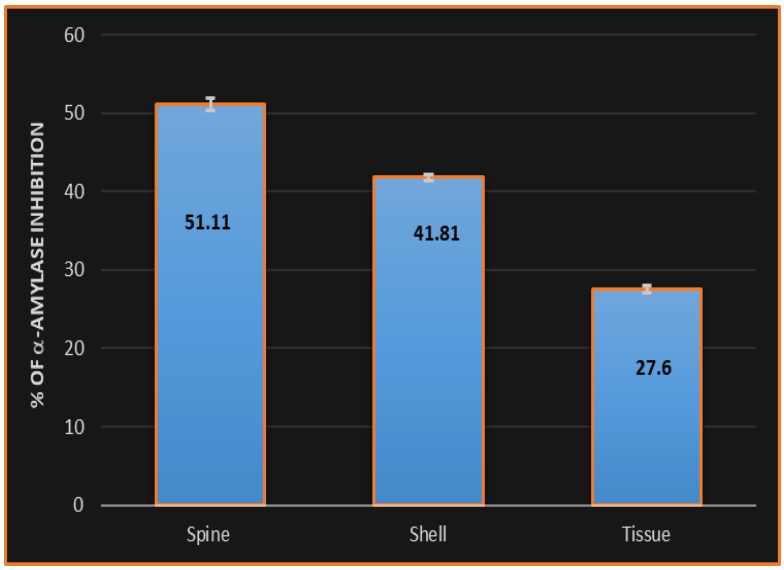


**Fig. 5. α-Amylase inhibitory activity of *Stomopneustes variolaris* spine, shell and tissue protein extracts**

## 4. CONCLUSION

The present study provides scientific evidence that *S. variolaris* can be used to treat diabetic symptoms. The diabetic properties of *S. variolaris* was assessed by in-vitro approaches such as effect of haemoglobin glycation, %GDRI, Glucose uptake by yeast cells and inhibition of alpha amylase. The observations of invitro studies concluded that *S. variolaris* protein extracts inhibit the haemoglobin glycation, thereby reduce the glycated product formation. The results of GDRI suggests that the glucose diffusion retardation is by delaying the glucose liberation from the starch thereby decrease glucose absorption and finally reduce the blood glucose levels. The invitro studies of glucose uptake revealed that *S. variolaris* protein extracts increase a substantial amount of glucose uptake by the yeast cells. All the results of invitro assays in the current study showed that the protein extracts of *S. variolaris* possess good antidiabetic activity. The present investigation has also opened a way for future research to develop potent formulation of drugs for diabetes.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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