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In vitro antidiabetic property of chitosan extracted from freshwater edible crab *Sartoriana spinigera* (Wood-Mason, 1871)

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Abstract- Alpha (α) amylase is the key hydrolysing enzyme involved in the digestion of carbohydrates. Retardation of starch digestion by inhibition of enzymes such as α-amylase plays a key role in the control of diabetes. Purpose of the study was to investigate *in vitro* antidiabetic property of chitosan extracted from carapace and chelate legs of freshwater edible crab *Sartoriana spinigera* using Amylase inhibition assay. The amylase inhibition assay was performed using the chromogenic DNSA method where chitosan was screened for its amylase inhibitory activity in a range of 10µg/mL-100µg/mL. IC_{50} value was calculated using Liner Regression method. The carapace chitosan and chelate legs chitosan was found to exhibit an inhibitory action on amylase enzyme up to 18.87% and 82.00% respectively at maximum concentration (100µg/ml). Acarbose was used as reference drug. Thus, it can be concluded that the chitosan extracted from carapace and chelate legs of *Sartoriana spinigera* has *in vitro* antidiabetic potential. Further studies can be carried out on molecular mechanism of amylase inhibition of chitosan extracted from *Sartoriana spinigera*, to yield an ecofriendly, cost-effective and easily accessible remedy for diabetes.

Key words: Amylase, IC_{50}, *Sartoriana spinigera,* Diabetes

INTRODUCTION

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the World.[1] In India, there are estimated 77 million people above the age of 18 years are suffering from diabetes (type 2) and nearly 25 million are prediabetics (at a higher risk of developing diabetes in near future).[2] The estimates in 2019 showed that 77 million individuals had diabetes in India, which is expected to rise to over 134 million by 2045. Approximately 57% of these individuals remain undiagnosed.[3] One of the effective methods to control diabetes is to inhibit the activity of alpha amylase enzyme which is responsible for the breakdown of starch to simpler sugars.[4] This is contributed by alpha amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycaemic individuals.[5] Some inhibitors in clinical use such as acarbose, miglitol, and voglibose produce serious side effects such as bloating, and abdominal discomfort.[6]

Chitin is a polysaccharide made up of β-(1→4) linked N-acetyl-D-glucosamine that is widely found in nature forming exoskeletons of crustaceans such as crabs, lobsters, krill and shrimps and insects as well as components of bacterial cell wall.[7] Chitin the precursor to chitosan was first discovered in mushroom by French Professor Henri Braconnot[8] in 1811. The application potential of chitosan, a deacetylated derivative of chitin, is multidimensional, such as in food and nutrition, biotechnology, material

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science, drugs and pharmaceuticals, agriculture and environmental protection, and recently in gene therapy too.[9]

Sartoriana spinigera a freshwater edible crab is abundantly found in water bodies of Jharkhand.[10] Although different body parts of crabs are used in ethnomedicine for the treatment of inflammatory and related disorders by tribal folk in Jharkhand.[11] Chitosan produced by marine sources have exhibited hypoglycemic effects but the use of exoskeleton of freshwater edible crab *Sartoriana spinigera* and its *in vitro* antidiabetic property have not been investigated. Hence, here an attempt made to know the *in vitro* anti diabetic activity of chitosan extracted from carapace and chelate legs of *Sartoriana spinigera* by using α-amylase enzyme inhibition assay models.

MATERIALS & METHODS

Extraction of chitosan: Chitosan was extracted following the method of Takiguchi (1991 a,b)[12,13] exoskeleton (carapace and chelate legs) powder was demineralized, deproteinized and deacetylated and finally extracted chitosan was confirmed by FTIR studies. Degree of deacetylation was calculated by graph obtained from FTIR-spectroscopy following the formula of Brugnerotto, J. *et al*, (2001)[14].

***In vitro* evaluation of antidiabetic property of chitosan (carapace and chelate legs) using α-Amylase inhibition assay[15]:**

The inhibition assay was performed using the chromogenic Dinitrosalicylic acid (DNS) method. The total assay mixture composed of 1440 mL, 1400 mL and 1350 mL of 0.05 M sodium phosphate buffer (pH 6.9), 50 mL of amylase and carapace chitosan and chelate legs chitosan samples at concentrations of 10 µg/mL, 50 µg/mL and 100 µg/mL were incubated at 37°C for 10 minutes. After pre-incubation, 500 mL of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for another 15 minutes. The reaction was terminated with 1.0 mL DNS reagent, placed in boiling water bath for 5 minutes, cooled to room temperature and the absorbance was measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS.

The liberated sugar was determined by the help of standard maltose curve and activities were calculated according to the following formula:

$$\underset{(\mu mol/ml/min)}{Enzyme\ activity} = \frac{\frac{conc.of\ maltose\ liberated}{mol.wt.of\ maltose} \times \frac{ml\ of\ enzymes\ used}{incubation\ time(min)}}{} \times dilution\ factor$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions.

The inhibitory property shown by the sample was compared with that of control and expressed as percent inhibition. This was calculated according to the following formula:

$$\%\text{Inhibition} = \frac{activity\ in\ presence\ of\ compound}{control\ activity} \times 100$$

Analysis of Acarbose as standard inhibitor:

Acarbose was used as a standard inhibitor and it was assayed as above-mentioned test sample concentrations. The assay method was similar to the above-mentioned procedure, instead of test samples, acarbose was added. The results were compared to that of test sample.

RESULTS

Extracted chitosan was confirmed by FTIR analysis. The FTIR spectrum of chitosan samples in the present study was obtained and effective peaks were compared with that of standard chitosan. Effective peaks of carapace chitosan were observed at 709.80, 875.68, 1026.13, 1489.76, 1658.78, 2885.51 and 3267.41 cm^{-1} and that of chelate legs chitosan were observed at 894.97, 1033.85, 1423.47, 1573.91, 1662.64 and 2881.65 cm^{-1}. The peaks observed in case of chelate legs chitosan and carapace chitosan out of 9 bands, 6 and 7 bands respectively were very similar to that of standard chitosan. The degree of deacetylation (DD%) of chitosan plays a significant role for determining the specific applications of chitosan. The degree of deacetylation of carapace chitosan and chelate legs chitosan obtained from *Sartoriana spinigera* was 98.190% and 80.156% respectively.

In the present study *in vitro* α-amylase inhibitory assay, carapace chitosan exhibited potent α-amylase inhibitory activity in a less dose dependent manner**.** % inhibitory activity of carapace chitosan at 10 µg/mL, 50 µg/mL and 100 µg/mL was found to be 4.63%, 10.75% and 18.87 % respectively (Table-2) with an IC_{50} value of 296.91 µg/mL. Chelate leg's chitosan exhibited potent α-amylase inhibitory activity in a dose dependent manner. % inhibitory activity of chelate legs chitosan at 10 µg/mL, 50 µg/mL and 100 µg/mL was found to be 34.14%, 65.49%

and 82.00% respectively (Table-1) with an IC_{50} value of 33.1957 µg/mL (Table-2). Acarbose was used as a standard drug. Acarbose at a concentration of 10-100 µg/mL showed α-amylase inhibitory activity from 43.41% to 100% respectively (Table-1) with an IC_{50} value of 1.69 µg/mL (Table 2). Chelate legs chitosan exhibited higher % inhibition against Amylase enzyme than carapace chitosan. % amylase inhibition for carapace chitosan was found to be 4.63% at 10 µg/mL and only 18.87% at 100 µg/mL whereas for chelate legs chitosan 34.14% at 10 µg/mL and 82.00% at 100 µg/mL.

Table 1- Estimated value of % Amylase inhibition of control, Acarbose and carapace chitosan and chelate legs chitosan.

Groups	Concentrations (µg/mL)	OD (540 nm)	Maltose liberated (µg)	Activity of Amylase (µmol/mL/min)	Amylase Activity (%)	Amylase Inhibition (%)
Control	10	0.570	44.92	0.012466	100.00	0.00
Acarbose	10	0.336	25.42	0.007054	56.59	43.41
	50	0.015	-1.33	-0.000370	0.00	100.00
	100	0.00	-2.58	-0.000717	0.00	100.00
Carapace chitosan	10	1.699	139.00	0.038578	95.37	4.63
	50	1.592	130.08	0.036103	89.25	10.75
	100	1.450	118.25	0.032819	81.13	18.87
Chelate leg's chitosan	10	0.386	29.58	0.008211	65.86	34.14
	50	0.217	15.50	0.004302	34.51	65.49
	100	0.128	8.08	0.002243	18.00	82.00

Table 2- LC_{50} values for *in vitro* amylase inhibition activity of Acarbose, carapace chitosan and chelate legs chitosan

Groups	IC_{50} value (µg/mL)
Acarbose	1.69
Carapace chitosan	296.91
Chelate leg's chitosan	33.19

DISCUSSION

In the present study, chelate leg's chitosan exhibited higher % inhibition against α-amylase enzyme than carapace chitosan. % amylase inhibition for carapace chitosan was found to be 4.63% at 10 µg/mL and only 18.87 % at 100 µg/mL whereas for chelate legs chitosan 34.14% at 10 µg/mL and 82.00% at 100 µg/mL. Ravi *et al.* (2012)[16] reported two mollusc species which had *α*-amylase inhibiting activity. Maximum inhibition observed by acetone extract of *Hemifusus pugilinus* was 72.23±0.44% at 50 µL concentration and highest inhibitory effect exhibited by acetone extract of *Natica didyma* was 51.23±0.44% at 50 µL concentration. But, the control drug (Acarbose) had significantly higher inhibitory action. According to Sudha *et al.* (2011)[17], 3 isopropanol plant extracts exhibited concentration dependent inhibition with IC_{50} values, viz., seeds of *Linum usitatissimum* (540 µg/mL), leaves of *Morus alba* (1440 µg/mL) and *Ocimum tenuiflorum* (8.9 µg/mL) against porcine pancreatic amylase. Acarbose as the standard inhibitor exhibited an IC_{50} (half maximal inhibitory concentration) value of 10.2 µg/mL. But in the present study IC_{50} value of chelate legs chitosan (33.1957 µg/mL) of α-amylase inhibition is higher than plant extract of *Linum usitatissimum* (540 µg/mL) and leaves extract of *Morus alba* (1440 µg/mL). Sindhu *et al.* (2013)[18] reported that the α-amylase inhibition assay of methanolic extracts of *Cinnamomum zeylanicum* (130.55 µg/mL), *Artocarpus altilis* (118.88 µg/mL), *Piper betel* (84.63 µg/mL) and *Artocarpus heterophyllus* (70.58 µg/mL) exhibited 50% α-amylase inhibition activity. But in the present study the IC_{50} value of α-amylase inhibition of chelate legs chitosan (33.1957 µg/mL) was higher than the plants *Cinnamomum zeylanicum* (130.55 µg/mL), *Artocarpus altilis* (118.88 µg/mL), *Piper betel* (84.63 µg/mL) and *Artocarpus heterophyllus* (70.58 µg/mL).

CONCLUSION

The above results indicated that chitosan extracted from exoskeleton of *Sartoriana spinigera* possess the inhibitory effect on α-amylase enzyme. This enzyme activities in the body are responsible for postprandial hyperglycemia by break down of dietary carbohydrates into glucose. It may lead to reduction in post prandial hyperglycemia in diabetic condition.

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