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Investigation of inhibition of protein denaturation assay by chitosan extracted from exoskeleton of *Sartoriana spinigera* (Wood-Mason, 1871)

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Abstract : Chitosan extracted from exoskeleton (carapace) of freshwater edible crab *Sartoriana spinigera* (Wood-Mason, 1871)[1] was assessed for *in-vitro* Protein (BSA) denaturation inhibition. The % Protein Denaturation Inhibitory activity of chitosan was tested at subsequent concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml and the present results exhibited a concentration dependent inhibition of protein (BSA) denaturation by the test extract i.e. 42.35±0.08, 49.41±0.9%, 58.82±0.67%, 69.41±0.60% and 83.53±1.03% respectively. Diclofenac Sodium was used as reference drug. 50 % Inhibitory Concentration (IC_{50}) values on protein (BSA) denaturation of Diclofenac sodium was 53.57(µg/ml) while IC_{50} value of the carapace chitosan was found to be 97.72 (µg/ml).

Keywords- *In vitro* Protein denaturation inhibition assay, *Sartoriana spinigera,* chitosan, IC_{50}

INTRODUCTION

Protein denaturation has been correlated with the formation of inflammatory disorders like rheumatoid arthritis, diabetes and cancer etc. Therefore, ability of substance to prevent the protein denaturation may also help to prevent the inflammatory disorders (Sangeetha G., Vidhya R., 2016)[2].

The most commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers (Tripathi K.D., 2008)[3], (Bennett P.N. & Brown M.J., 2005)[4].

Natural resources have contributed significantly the development of modern medicine. Recently traditional folk medicine worldwide is being re-evaluated by extensive research on different plant and animal species and their active therapeutic principles. The rich wealth of flora and fauna can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of traditional medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Although different parts of crabs are used in ethnomedicine for the treatment of inflammatory and related disorders by tribal folk in Jharkhand but the use of carapace chitosan extracted from *Sartoriana spinigera* and its anti-arthritic property have not been pharmacologically evaluated. Hence the present study undertaken to evaluate the anti-arthritic activity of carapace chitosan of *Sartoriana spinigera* by *in vitro* method.

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MATERIALS & METHODS

a) Collection of samples: Live specimens of freshwater crab *S. spinigera* were purchased from local market of Ranchi, Jharkhand and kept in aquarium in research laboratory of P.G. Department of Zoology, Ranchi University.

b) Extraction of chitosan: After 7 days acclimatization, crabs were narcotized and carapace were removed, washed, cleaned thoroughly and dried in hot air oven at 37°C. Then they were grinded to fine particles. Chitosan was extracted using the chemical process (Takiguchi, 1991)[5]. The demineralization process was done by using hydrochloric acid. The filtrate was soaked in 4% NaOH for 24 hrs at 80°C for deproteinization. The powdered form of Chitin obtained was deacetylated with 50% NaOH heated at 110°C and the filtrate was dried to obtain Chitosan.

c) Characterization of chitosan: - Samples of chitosan extracted from carapace of crab *S. spinigera* were sent to Central Instrumentation Facility, BIT Mesra for FTIR spectroscopy**.** Degree of deacetylation was calculated by graph obtained from FTIR-spectroscopy following the formula Brugnerotto, J. *et al*., (2001)[6]: **Degree of deacetylation (DD %)** = 100-Degree of acetylation. **Degree of acetylation** (%) = 31.92 × A1320 /A1420 – 12.20, where, A1320 and A1420 represents the absorbance at 1320 and 1420 cm^{-1} respectively.

d) Evaluation of *in vitro* anti-arthritic activity (R. Lavanya *et al.*, 2010)[7]:

♦ **Assay method:** In this model assessment of anti-arthritic activity of chitosan was studied by using inhibition of protein (BSA) denaturation technique which was done according to R. Lavanya *et al*., (2010)[7]. The test sample as well as standard drug solutions i.e. chitosan of carapace were taken in different concentrations- 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml and assayed in triplicates. The reaction mixture 0.5ml consisted of 0.45ml bovine serum albumin (BSA) of 5% aqueous solution + 0.05ml of 50-250µg/ml of extract. Distilled water instead of extracts with above mixture was used as a negative/test control and positive/product control 0.5ml consisted distilled water and test solution. The pH of all the prepared solutions were adjusted to 6.3 with the help of 1N HCl. Diclofenac sodium was used as standard drug. All the solutions were incubated at 37^0 C for 20 minutes. After that, all the solutions were kept at 57^0 C for 3 minutes. And after cooling, 2.5 ml of 0.1 M phosphate buffer was added to the all test tubes. Absorbance was read at 416nm.

♦ Percent (%) inhibition of Protein (BSA) denaturation was calculated using the formula: -

[100 - {(OD of TEST SOLUTION - OD of PRODUCT CONTROL) / OD of TEST CONTROL} × 100]

STATISTICAL ANALYSIS:

All *in vitro* assays data signify the mean ± standard deviation of triplicates. The extract/drug concentration for 50% inhibition (IC_{50}) was determined form the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS & DISCUSSIONS

FT-IR studies: The FT-IR spectrum of chitosan in the present study was obtained and effective peaks were compared with that of standard chitosan. The FT-IR spectrum of the standard chitosan showed nine major peaks at the ranges of 752, 893, 1022, 1421, 1552, 1643, 2865, 2921 and 3283 cm^{-1} (Puvvada *et al*., 2012)[8] peaks of chitosan sample extracted from *Sartoriana spinigera* were compared. Only 7 out of 9 peaks were nearly similar with that of standard 709.80, 875.68, 1026.13, 1489.76, 1658.78, 2885.51 and 3267.41 cm^{-1}. **DD (%):** Degree of deacetylation affects the chemical, physical and biological properties of chitosan such as adsorption, covalent linking, encapsulation (Puvvada *et al*. 2012)[8]. Degree of deacetylation (DD %) of the chitosan was found 98.19%, it is higher than reported by Puvvada *et al*. (2012)[8] in which degree of deacetylation of chitosan of shrimp waste was 89.79%. The obtained chitosan was found to be soluble in 1% acetic acid and the pH was found to be 8. FT-IR studies confirmed that the obtained product was chitosan.

Table 1: Percentage inhibition of protein (BSA) denaturation by the carapace chitosan sample and Diclofenac sodium

Concentrations (µg/ml)	% inhibition of Protein (BSA) Denaturation	
	Diclofenac sodium	Carapace Chitosan of *S. spinigera*
50	54.12±0.85	42.35±0.85
100	55.29±0.75	49.41±0.9
150	61.18±1.07	58.82±0.67
200	71.76±0.60	69.41±0.60
250	87.06±1.70	83.53±1.03

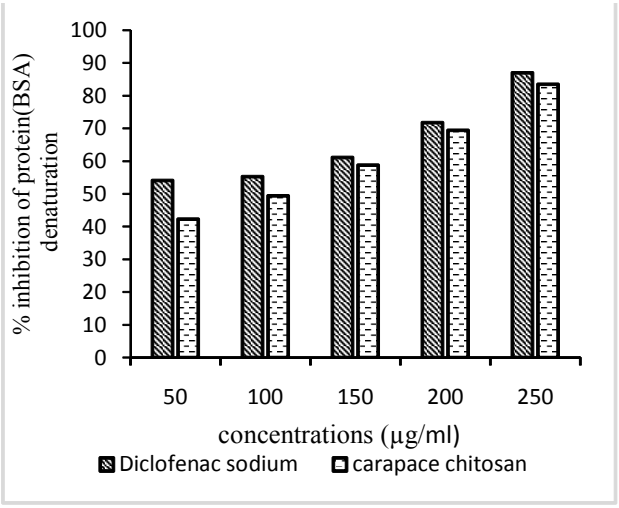

Fig 1: - % inhibition of protein (BSA) denaturation by Diclofenac sodium and carapace chitosan.

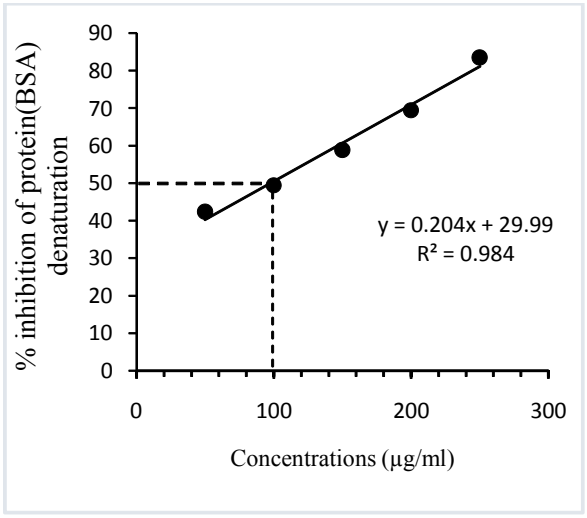

Fig 2: - 50% inhibitory concentration (IC_{50}) value for carapace chitosan was 97.72 (µg/ml)

Table 2: - 50 % inhibitory concentration (IC_{50}) values on protein (BSA) denaturation

Groups	IC_{50} (µg/ml)
Standard (Diclofenac sodium)	53.57
Test (Carapace chitosan)	97.72

The present investigation showed % inhibition of protein (BSA) denaturation by sample as well as Diclofenac sodium drug. Protein (BSA) denaturation inhibition assay was done with five different (50µg, 100µg, 150µg, 200µg and 250µg) concentrations of chitosan sample as well as standard drug. An *in-vitro* anti-inflammatory study revealed that Chitosan sample with 50µg showed 42.35±0.85% inhibition and it gradually increased (Fig. 1) to 49.41±0.9%, 58.82±0.67%, 69.41±0.60%, 83.53±1.03% inhibition for 100µg, 150µg, 200µg and 250µg respectively whereas in case of Diclofenac sodium at 50µg showed 54.12±0.85% and at 250µg showed 87.06±1.70 % inhibition. The results are summarized in Table (1), the present findings exhibited a concentration dependent inhibition of protein denaturation by the test extract throughout the concentration range of 50µg, 100µg, 150µg, 200µg and 250µg/ml. Diclofenac sodium (at the concentration range of 50µg, 100µg, 150µg, 200µg and 250µg/ml) was used as the reference drug which exhibited highest inhibition of protein denaturation. This was further confirmed by comparing their IC_{50} values. R. Lavanya *et al.* (2010)[7] reported that methanolic extracts of *Anisomeles malabarica* L. at a dose of 250µg/ml protein (BSA) denaturation was found to be 97.47%. While in this study the chitosan showed 83.53±1.03% at 250µg/ml. In this study the chitosan showed 83.53±1.03% at 250µg/ml concentration which is higher than the report of Darsan B. Menon *et al.* (2011)[9], they reported that the percentage inhibition of protein (BSA) by methanolic extract of the stem of *Plectranthus hadiensis* and standard Diclofenac, at 1mg/mL concentration, was 86.10% and 92.93% respectively. Srividya and Chandra (2015)[10] reported Inhibition of protein (albumin) denaturation at 200µg/ml was found to be highest in *Phyllanthus emblica* followed by *Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta, Artocarpus hirsutus* and the values were 79.01±3.07%, 65.75±2.53%, 51.57±2.83%, 33.71±1.10%, 28.73±2.53% and 28.00±1.94% respectively. Percentage inhibition value for the standard diclofenac sodium was found to be 92.64±2.30%. **IC_{50}: -** in this present study 50 % Inhibitory Concentration (IC_{50}) values on protein (BSA) denaturation of Diclofenac sodium was 53.57(µg/ml) while IC_{50} value of the carapace chitosan was found to be 97.72 (µg/ml) (Table-2), which is lesser than the *in vitro* protein denaturation assay of fresh water edible snail *Bellamya*

bengalensis reported by Anjan *et al.* (2015)[11] ,they found that the 50% Inhibitory Concentration (IC_{50}) of extrapallial fluid of the mollusca *Bellamya bengalensis* on protein denaturation was 87.56 µl/ml .

CONCLUSION

Carapace chitosan extracted from *Sartoriana spinigera* and its *in vitro* anti-arthritic property have not been pharmacologically evaluated. Thus, on the basis of the data obtained in this study it may be concluded that the chitosan extracted from carapace of *Sartoriana spinigera* contains the capacity to inhibit the protein denaturation *in vitro* and confirms its anti-inflammatory (anti-arthritic) properties. Further studies may require for its mechanism of action.

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