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Comparative Cytotoxicity Study of Six Bioactive Lectins Purified from Pondweed (*Potamogeton nodosus* Poir) Rootstock on Brine Shrimp

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Six bioactive lectins were purified from the rootstock of Pondweeds (*Potamogeton nodosus* Poir) by conventional chromatographic methods. They showed cytotoxic effect in brine shrimp (*Artemia salina* L.) lethality bioassay. The LD-50values of PNL-1, PNL-2, PNL-3, PNL-4, PNL-5 and PNL-6 were found to be 10.76, 7.03, 17.25, 10.52, 19.60 and 20.1 μg mL^{-1}, respectively which implied that they have significant uses specially of PNL-2. Experimental results revealed that PNL-2 and PNL-4 were more cytotoxic than other lectins.

Key words: Rootstock, *Potamogeton nodosus* poir, lectins, cytotoxicity

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INTRODUCTION

Lectin, as mentioned above, is a group of glycoprotein that bind sugar specifically and reversibly and possesses the unique ability to agglutinate erythrocytes and other types of cells (Boyd, 1970). Lectins are widely available in nature as they found in animals, insects, plants and microorganisms (Linear *et al*., 1986; Lis and Sharon, 1986; Lin *et al*., 1981; Sharon and Lis, 1989). Lectins are widely distributed in the plant kingdom and are also referred to as phytohemagglutinin. The term phytolectin has been proposed in order to distinguish those lectins which are found in plants from those which are of animal or microbial origin.

Potamogeton nodosus Poir (Synonym: Potamogeton nodosus Poir, Local Ghechu) is a protein rich, submerged aquatic herb with creeping rootstock which belongs to the family Potamogetonaceae and genus Potamogeton (Khan and Halim, 1987). The plant mainly grows in pools, marshy lands, old ponds and calals of Indian subcontinent as well as in Bangladesh, Sikkim, Burma, Malaya, Srilanka and Malaysia region (Haq, 1986). The plant is of little economic value and in Ayurveda, it is claimed to be effective against cough, tuberculosis, acne, cancer, diarrhoea, dysentery, jaundice etc. (Biswas and Ghosh, 1977).

Plant materials contain mostly glycoprotein that are toxic in nature. Keeping this view in mind, we have first time purified six bioactive lectins (a class of glycoprotein) from the rootstock of ghechu and also trying researches to obtain clear information about the nature of the purified lectins.

MATERIALS AND METHODS

Collection of *Potamogeton nodosus* rootstock: The rootstocks of *Potamogeton nodosus* Poir were collected from the marshy lands of Tanore Upazilla under Rajshahi district, Bangladesh. After collection, the rootstocks were preserved in a desiccator at 4°C.

Extraction and purification: The sliced rootstocks were taken in a mortar and pounded uniformly into fine powder. Fat free meal was prepared by adding pre-cooled petroleum ether (40-60°C) into the powder and homogenized uniformly with the homogenizer. The homogenate was centrifuged at 8000 g for 15 min at 4°C, the precipitates was collected, dissolved in minimum volume of water and dialyzed against water and Tris-Hcl buffer pH 8.4. After dialysis, it is again centrifuged at 8000 g for 8 min and the supernatant was collected and used for purification of lectins.

Four *Potamogeton nodosus* lectins (PNL) i.e., PNL-1, PNL-3, PNL-5 and PNL-6 were purified by gel filtration of 100% ammonium sulfate saturated crude extract on Sephadex G-50 following ion-exchange chromatography on DEAE-cellulose while two other lectins i.e., PNL-2 and PNL-4 were purified by further chromatography on CM-cellulose. The purity of the lectins were checked by SDS-PAGE electrophoresis.

Cytotoxicity bioassay: Brine shrimp lethality bioassay (Persoone, 1980; Mayer *et al*., 1982; Mclaughlin and Anderson, 1988; Mclaughlin, 1990) is a recent development in the assay procedure of bioactive compounds which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g., anticancer, antiviral, insecticidal, pesticidal, AIDS etc.) of the compounds. Here *in vivo* lethality, a simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. Cytotoxicity bioassay of the purified lectins were carried out using brine shrimp nauplii eggs (*Artemia salina* L.). Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In the other side of the tank, a light source was placed in order to attract the nauplii. Two days were allowed for the hatching of all the eggs and sufficient maturation of the nauplii for experiment described by Mayer *et al.* (1982).

The test lectins were dissolved in dimethyl sulphoxide (DMSO) and five grade doses 1.6, 3.2, 6.4, 12.8 and 25.6 μg mL^{-1}, respectively were used for 5 mL seawater containing 100 brine shrimp nauplii in each group. Three vials were used for each concentration and the control was used containing 100 nauplii in 5 mL of DMSO and seawater. After 24 h incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. From these data, the percentage of mortality of the nauplii was calculated for each concentration and the LC_{50} values were determined from the log dose response curve (Goldstein and Kalkan, 1974) and also by using probit analysis as described by Finney (1971).

RESULTS AND DISCUSSION

All the six lectins showed a significant cytotoxic activity in the brine shrimp lethality bioassay (Table 1). Mortality of the nauplii was noticed in the experimental groups at the same time the control group remained unchanged. The number of survived nauplii in each vial was counted and the results were noted. From these data the percent mortality of the brine shrimp was calculated

Table 1: Brine shrimp lethality bioassay of lectins purified from pondweeds

Sample	Dose	log dose	Mortality (%)	Corr (%)	Emp probit	Expt probit	Wrk probit	Weight	Final probit
PNL-1	1.6	0.2041179	20	20	4.16	4.135998	4.17	47.1	4.139904
	3.2	0.5051448	28	28	4.42	4.452999	4.42	55.80	4.45275
	6.4	0.8061716	40	40	4.75	4.77	4.74	61.6	4.765597
	12.8	1.107199	55	55	5.13	5.087	5.125	63.7	5.078443
	25.6	1.408225	65	65	5.39	5.404001	5.375	60.1	5.39129
PNL-2	1.6	0.2041179	30	30	4.48	4.398	4.49	53.2	4.396008
	3.2	0.5051448	35	35	4.61	4.681	4.605	60.1	4.678851
	6.4	0.8061716	44	44	4.85	4.964	4.84	63.4	4.961695
	12.8	1.107199	64	64	5.36	5.247001	5.384	62.7	5.244539
	25.6	1.408225	70	70	5.52	5.530001	5.5	58.1	5.527382
PNL-3	1.6	0.2041179	20	20	4.16	4.126	4.17	47.1	4.130446
	3.2	0.5051448	26	26	4.36	4.381	4.362	53.2	4.383928
	6.4	0.8061716	35	35	4.61	4.636	4.605	60.1	4.637411
	12.8	1.107199	45	45	4.87	4.891001	4.890	62.7	4.890893
	25.6	1.408225	57	57	5.18	5.146001	5.165	63.4	5.144376
PNL-4	1.6	0.2041179	25	25	4.33	4.234	4.32	50.30	4.217438
	3.2	0.5051448	30	30	4.48	5.519	4.46	58.1	4.505436
	6.4	0.8061716	38	38	4.69	4.804	4.708	62.7	4.793434
	12.8	1.107199	52	52	5.05	5.089	5.05	63.7	5.081432
	25.6	1.408225	68	68	5.47	5.374	5.448	61.6	5.36943
PNL-5	1.6	0.2041179	15	15	3.96	4.02	3.955	43.9	4.022636
	3.2	0.5051448	25	25	4.33	4.292	4.32	50.30	4.292971
	6.4	0.8061716	35	35	4.61	4.564	4.6	58.1	4.563306
	12.8	1.107199	45	45	4.87	4.836001	4.890	62.7	4.833641
	25.6	1.408225	52	52	5.05	5.108001	5.04	63.4	5.103976
PNL-6	1.6	0.2041179	25	25	4.33	4.414	4.33	55.80	4.411319
	3.2	0.5051448	36	36	4.64	4.574	4.628	58.1	4.572556
	6.4	0.8061716	42	42	4.80	4.734	4.792	61.6	4.733793
	12.8	1.107199	46	46	4.90	4.894	4.916	62.7	4.895029
	25.6	1.408225	50	50	5.00	5.054001	5.00	63.7	5.056267

Table 2: Toxicity levels of the lectins purified from pondweeds

Sample	Regression equation	Chi-square value	LOG LD-50	LD-50	95% Conf. limits
PNL-1	Y = 3.927771+1.039265 X	0.2967148	1.031719	10.75769	8.118416 to 14.25498
PNL-2	Y = 4.20422+0.9395951 X	2.999058	0.8469398	7.029748	8.118416 to 14.25498
PNL-3	Y = 3.958566+0.8420597 X	0.1901894	1.23677	17.24924	11.20608 to 26.55132
PNL-4	Y = 4.022155+0.9567182 X	1.550083	1.022083	10.52162	7.791081 to 14.20913
PNL-5	Y = 3.839329+0.8980431 X	0.7745476	1.292445	19.60852	12.71328 to 30.24348
PNL-6	Y = 4.301988+0.5356231 X	0.9862089	1.303177	20.09912	9.684488 to 41.71359
Gallic acid (Reference)		1.25		4.53	3.33 to 6.15

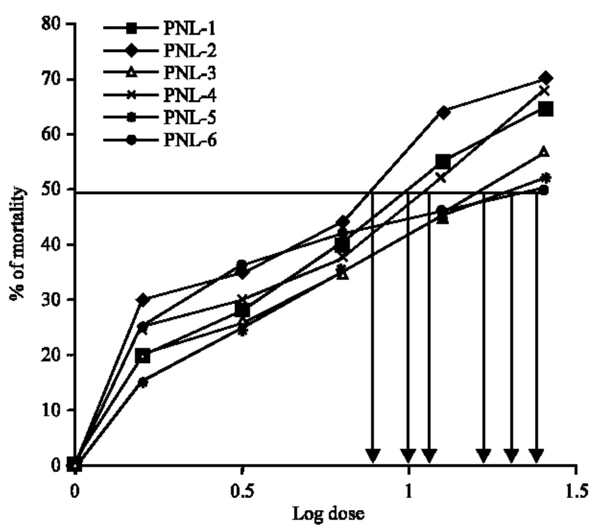

Fig. 1: Determination of LD-50 from log dose response curve

for every concentration of each sample. The mortality rate of brine shrimp nauplii was found to increase with the increase in concentration of sample and a plot log concentration versus percent mortality on graph paper gave an almost linear correlation (Fig. 1). The LD-50 (concentration/dose at which 50% mortality of the nauplii occurs) values were obtained after probit analysis and the values for the lectins, PNL-1, PNL-2, PNL-3, PNL-4, PNL-5 and PNL-6 were found to be 10.75769, 7.029748, 17.24924, 10.52162, 19.60852 and 20.09912 μg mL^{-1}, respectively (Table 2). It is evident from the results of brine shrimp lethality testing that the LD-50value of PNL-2 was the lowest (7.029748 μg mL^{-1}) indicating its higher toxicity. On the other hand these LD-50values of PNL-1, PNL-3, PNL-4, PNL-5 and PNL-6 were higher compared to PNL-2. The potency of the lectins was of the following order PNL-2> PNL-4> PNL-1> PNL-3> PNL-5> PNL-6. The finding is very similar with that of mulberry seed lectin (MSL-1) (Hossain *et al*., 2002). These LC_{50} values may be used in determination of the Therapeutic Index (TI) of the respective compounds.

CONCLUSION

Our experiment showed that LD-50 values of the six lectins were around the toxic level. In the present investigation we can conclude that the lectins PNL-1, PNL-2 and PNL-4 may have anticancer and antitumour activity as they showed more cytotoxic activity and further investigations are needed to confirm their bioactivity by applying on human cancer cell and bacteria to stablish these as anticancer and antitumour agents which may explore as potent chemotherapeutic agent(s) in modern clinical trials.

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