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Effect of Cisplatin and 5-Fluorouracil on Total Protein Contents in Fresh Water Bivalve Parreysia Corrugata.

KEYWORDS: Cisplatin, 5-Fluorouracil, Anticancer drugs, chemoprevention, Cytotoxicity.

Mr. Gawali R.D.
K. J. Somaiya college of Arts, Commerce and Science, Kopargaon, Dist. Ahmednagar, 423601(M.S.), India,

Dr. Zambare S.P
Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431004 (M.S.), India.

ABSTRACT *Cisplatin and 5-Fluorouracil are potent and valuable anticancer drugs widely used for chemotherapy against solid tumors. These drugs exhibits effective chemoprevention in cancer therapy and also lead to several manipulations and cytotoxicity. In present toxicity studies, sub-lethal doses of cisplatin and 5-fluorouracil (LC50/10 for 96 hours) were given to an experimental model, the fresh water bivalve Parreysia corrugata for 30 days. The total protein contents were estimated from different tissues of control and treated bivalves by Lowry's method. It was found that the total protein contents were decreased in tissues of experimental bivalves with increase in period of exposure to these anticancer drugs. It was also observed that the decrease in amount of total protein content in tissues was more in cisplatin treated bivalves than that of 5- fluorouracil treated bivalves.*

Introduction:

Most of the aquatic organisms are exposed to various toxicants in polluted environment that leads to chronic toxicity of the pollutants. Among the animals there is protective mechanism of the body to resist and combat the toxic effects of toxicants like heavy metals and their derivatives. It is observed that some biochemical alterations occurring in the body gives the alarming indications of stress condition. The trace metals like mercury, cadmium, lead are known to be non bio-degradable and highly toxic to most organism. The toxic property of some metal complexes is useful in treatment of dreaded diseases like different types of cancers. Similarly there are some competitive inhibitors of the normal biological molecules which are readily metabolized and thus make the synthesized biomolecule inactive. Though such substances are harmful, man has used them in the treatment of diseases; however they have many serious side effects which need to be studied with some biological systems. Due to physiological differences, certain species accumulate these metals faster than others (Clark *et al.*, 2001). Metals are known to decrease the energy level by interfering with the metabolic pathway (Torreblanca *et al.*, 1992). Pollutants including heavy metals may alter cellular functions, ultimately affecting physiological and biochemical mechanisms of animals (Radhakrishnan *et al.*, 1991), due to their ability to form complexes with ligand (Vallee and Ulmer, 1972). It has been observed that heavy metals can cause biochemical alterations such as inhibition of enzymes, metabolic disorder, genetic damage, hypertension and cancer (Underwood, 1971; Lucky and Venugopal, 1977).

Cisplatin, cis-diammine dichloroplatinum II (cis-DDP), the platinum containing coordination complex is an effective antitumor agent used in the treatment of wide variety of human malignancies (Gottieb and Derwinko, 1975; Prestayko *et al.*, 1979 and Rozenewig *et al.*, 1977). The antitumor activity is to be considered due to its ability to bind guanine residue in DNA conformation and inhibition of DNA synthesis (Coven *et al.*, 1979). Cisplatin is very effective anticancer drug widely used in the treatment of the bladder, testis, ovary and other solid tumors (Broach *et al.*, 1987 and Hamers *et al.*, 1991).

5-fluorouracil (5-FU), itself has only modest anticancer activity but has been shown to be a very effective target for biochemical modulation. Biochemical modulation is a special type of combination chemotherapy which aims to selectively improve the therapeutic index by increasing the antitumor effect and protecting against toxic side effects. 5-Fluorouracil remains the mainstay of treatment for advanced gastric cancer and no standard chemotherapy regimen exists. Combination of irinotecan with folinic acid and infusional, 5-fluorouracil have shown good efficacy with acceptable toxicity as hematologic toxicity (anemia, neutropenia and leucopenia), non-hematologic (nausea/vomiting) and diarrhea in patients with metastatic colorectal cancer (Kunz, 1998). The efficacy of cisplatin therapy is greatly reduced by the development of intrinsic and acquired cisplatin resistance, which is very common in lung and ovarian cancer. One method used to combat cisplatin resistance has been the application of combination chemotherapy. For example cisplatin and 5-fluorouracil are synergistic when used in combination and has had some success in the treatment of lung and ovarian cancer (Johnson *et al.*, 1996).

The present study will be useful to develop the simple model for the screening of the anticancer drugs and their effects at the primary level. This study can also help us to compare effectiveness and side effects of various anticancer drugs.

Material and methods:

The fresh water bivalves, *Parreysia corrugata* were collected from Girna lake area near Jamda (Latitude 20^0 33'N, Longitude $75^0$10'E, 352 m MSL) which is 14 km away from Chalisgaon, District Jalgaon of Maharashtra State. Bivalves were collected and brought to laboratory in aerated container. They were maintained in a glass aquarium containing dechlorinated water (PH 7.0- 7.5) for 3- 4 days and acclimatized to laboratory conditions at 21^0C- 26^0C temperature. The water in aquarium was changed regularly after every 24 hours. After acclimatization, healthy medium sized bivalves were selected from the aquarium and used for experiments. The acclimatized bivalves, *Parreysia corrugata* were divided into three groups, with equal number of

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animals. They were kept in separate aquarium for 30 days. Bivalves from one of the three groups were not exposed to anticancer drugs and were maintained as control. Out of remaining two groups, one was treated by predetermined sub lethal concentration (LC_{50}/10 value of 96 hours) of cisplatin (1.007 ppm) and other group was treated by chronic concentration (LC_{50}/10 value of 96 hours) of 5- fluorouracil (4.078 ppm). On 10th, 20th and 30th day of exposure, bivalves from each experimental group were sacrificed and mantle, foot, gonads, digestive glands and gills were removed. These tissues were dried in oven at 60°C- 70°C till constant weight was obtained and blended into dry powder.

10 mg of dry powder was homogenized in small amount of 10% tricarboxylic acid and the homogenate was diluted to 10 ml by 10 % tricarboxylic acid. Then it was centrifuged at 3000 rpm for 15 minutes. The supernatant was removed and protein precipitate at the bottom of centrifuge tubes was dissolved in 10 ml 1.0 N NaOH solution. Total protein components of these dry powders were estimated by Lowry's method (Lowry *et al.*, 1951). The optical density of blue color developed was read at 530 nm on a colorimeter. The blank was prepared in same way by using 1 ml distilled water instead of protein extract.

The protein contents in different tissues were calculated referring to standard graph values and it is expressed in terms of mg protein /100 mg of dry tissue. The Bovine serum albumen was used as a standard protein.

Results and Discussion:

The values given in the Table indicate changes in protein levels of various tissues of *Parreysia corrugata* on chronic exposure to cisplatin (1.007 ppm) and 5-fluorouracil (4.078 ppm) for 10, 20 and 30 days. Protein level of different tissues of *Parreysia corrugata* on chronic exposure to cisplatin and 5-fluorouracil (5-FU) were decreased significantly ($p<0.05$) in mantle, foot, gonads, digestive glands and gills of experimental bivalves as compared to those of control bivalves. The (-) values given in table indicates percentage decrease of protein contents in treated bivalves than those of control group. It was also observed that the decrease in total protein content in various tissues of cisplatin treated bivalves was found to be more than that of 5-fluorouracil treated bivalves. Thus the cisplatin was found to be more toxic than that of 5-fluorouracil.

The decreased protein contents may be due to destruction of cells or necrosis of cells and may be followed by failure of protein synthesis machinery (Bradbury *et al.*, 1987). Different metal ions have been confirmed to accelerate free radical reaction in vitro (Quinlan, 1988).

Proteins plays significant role in cellular metabolism. All enzymes are proteins and control sub cellular functions. In metabolism of proteins many enzymes, coenzymes, intermediate proteins and amino acids involved are studied in many animals (Sekeri *et al.*, 1968). The nephrotoxicity, ototoxicity and neurotoxicity of cisplatin may be due to reactions with cellular molecules other than DNA (Deckam, 1995). Zeolite in catfish, *Heteropneustes fossilis* reduced nucleic acid and protein contents in exposed fish (James and Sampath, 2000). Post mortem studies have proposed oxidative injury by oxidative damage to proteins, lipids and DNA (Agar and Durham, 2003).

Table: Total Protein contents in different tissues of *Parreysia corrugata* on exposure to chronic dose of Cisplatin and 5-Fluorouracil.

Sr. No.	Tissue	Exposure to	10 Days	20 Days	30 Days
1	Mantle	Control	55.08 ± 1.828	54.12 ± 3.389	54.12 ± 2.366
		Cisplatin (1.007 ppm)	51.42 ± 1.802* (-7.318)	50.07 ± 1.981* (-7.483)	48.71 ± 0.708* (-9.996)
		5FU (4.078 ppm)	54.12 ± 1.090* (-2.451)	51.42 ± 2.637* (-4.989)	49.60 ± 1.816* (-8.352)
2	Foot	Control	60.88 ± 2.953	59.53 ± 1.798	58.18 ± 1.307
		Cisplatin (1.007 ppm)	55.48 ± 1.802* (-8.870)	52.77 ± 0.708* (-11.356)	46.01 ± 3.389* (-20.918)
		5-FU (4.078 ppm)	56.83 ± 2.670* (-6.652)	54.12 ± 2.757* (-9.088)	52.76 ± 1.414* (-9.316)
3	Gonads	Control	56.83 ± 2.953	55.48 ± 2.365	54.11 ± 3.389
		Cisplatin (1.007 ppm)	51.42 ± 1.803* (-9.520)	47.35 ± 3.050* (-14.654)	40.59 ± 0.708* (-24.986)
		5-FU (4.078 ppm)	52.77 ± 2.956* (-7.144)	50.07 ± 1.089* (-9.751)	47.36 ± 2.403* (-12.474)
4	Digestive glands	Control	59.53 ± 1.798	58.18 ± 3.766	56.83 ± 0.894
		Cisplatin (1.007 ppm)	54.12 ± 1.802* (-9.088)	50.06 ± 2.290* (-13.956)	44.65± 2.957* (-21.432)
		5-FU (4.078 ppm)	55.47 ± 2.365* (-6.821)	51.42 ± 2.404* (-11.619)	48.71 ± 0.722** (-14.288)
5	Gills	Control	50.07 ± 2.540	48.71 ± 1.414	47.35 ± 2.503
		Cisplatin (1.007 ppm)	47.35 ± 1.803* (-5.432)	43.30 ± 1.310* (-11.106)	35.18 ± 3.050* (-25.702)
		5FU (4.078 ppm)	48.71 ± 1.225* (-2.716)	46.01 ± 3.390* (-5.543)	37.88 ± 2.404* (-20.00)

(Values are expressed in mg / 100 mg of dry weight of tissues, Values are mean ± S.D. of five observations, (-) indicates % decrease over control, Significance of t-test at *p<0.05, **p<0.01, ***p<0.001).

Although higher doses of cisplatin are more effective for the suppression of cancer, high dose therapy produces several irreversible renal dysfunction, ototoxicity and neuropathy (Gandara *et al.*, 1991, Hamers *et al.*, 1993, Wolfgang *et al.*, 1994 and Rybak *et al.*, 1995). A number of therapeutic agents have been evaluated experimentally and clinically against cisplatin induced nephrotoxicity but none of them proved to be clinically effective as a complete protective agent. Oxidative stress has been reported in the cisplatin induced nephrotoxicity. Therefore, treatment with antioxidants was effective to repair the damage (Cetin *et al.*, 2006). The earlier experimental findings have suggested that the free radicals and reactive oxygen species are involved in cisplatin induced renal damage due to the depletion of growth stimulating hormone concentration and antioxidant enzyme activities in the kidneys (Ajith *et al.*, 2002 and Cetin *et al.*, 2006). The decrease in activ-

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ity of the antioxidant enzymes may be also involved in oxidative stress observed in cisplatin treated rats (Koc *et al.*, 2005 and Ajith *et al.*, 2007). Reduction in DNA contents in various tissues in fresh water bivalve *Cobicula striatella* was reported when exposed to chronic dose of 5-fluorouracil (Bhosale and Zambare, 2011).

Conclusion:

Protein contents are found to be significantly decreased in mantle, foot, gonads, digestive glands and gills of experimental bivalves, *Parreysia corrugata* on exposure to cisplatin and 5-fluorouracil as compared to the control group of bivalves.

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