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Effect of Cisplatin and 5-Fluorouracil on Ascorbic acid contents in fresh water bivalve Parreysia corrugata.

KEYWORDS: Cisplatin, 5-Fluorouracil, Anticancer drugs, chemoprevention, Ascorbic acid.

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ABSTRACT *Cisplatin and 5-Fluorouracil are potent and valuable anticancer drugs widely used for chemotherapy against solid tumours. 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 fresh water bivalves Parreysia corrugata for 30 days. The ascorbic acid contents were estimated from different tissues of control and experimental bivalves by Roe's method. It was found that ascorbic acid contents were decreased in tissues with increase in period of exposure to anticancer drugs in experimental bivalves. It was also observed that decrease in ascorbic acid contents in different tissues were more in cisplatin treated bivalves than that of 5- fluorouracil treated bivalves.*

Introduction

All aquatic organisms are exposed to various toxicants in polluted environment and leads to chronic toxicity of the pollutants. Among the animal 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. 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).

Ascorbic acid (Vitamin C) is a water soluble vitamin. It plays an important role in the human health and disease. It is widely used in mega doses to cure various diseases from common cold to cancer. It is strong reducing agent. Ascorbic acid undergoes oxidation to form dehydroascorbic acid. Oxidation of ascorbic acid is rapid in presence of copper hence vitamin C plays the role of a coenzyme in hydroxylation of proline and lysine while protocollagen is converted into the collagen .Ascorbic acid plays a very important role in tissue synthesis and growth processes and thus mediates rapid tissue repair in trauma and abnormal condition. L-ascorbic acid by virtue of possessing reducing properties is known to act radio protective agent in several tissue including reproductive organ by preventing radiation induced oxidation (Chinoy and Garg, 1978). The alteration in ascorbic acid contents due to the impact of pollutants in *Corbicula striatella* was reported by Zambare (1991). Ascorbic acid plays vital role as an antioxidant that serves the protective function against oxidative damage in tissues. The antioxidant property of ascorbic acid helps to prevent free radical formation from water soluble molecules, which may cause cellular injuries and disease. The free radical scavenging property of L- ascorbic acid is responsible for reducing genotoxic damage (Edgar, 1974).

Cisplatin, cis-diammine dichloroplatinumII (cis-DDP), platinum containing coordination complex is an effective antitumour 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 antitumour 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 *I*., 1991). Reduction in DNA contents in various tissues in fresh water bivalve *Cobicula striatella* was reported whenexposed to chronic dose of 5-fluorouracil (Bhosale and Zambare, 2011).

The mechanism of action of 5-fluorouracil (5-FU) has been associated with inhibition of thymidylate synthatase and incorporation of 5-FU into RNA and DNA, but limited data is available in human tumor tissue for the latter (Noordhuis *et al*., 2004). 5-fluorouracil, 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 antitumour effect and protecting against toxic side effects. 5-Fluorouracil remains the mainstay of treatment for advanced gastric cancer and no standard chemotherapy regimen exists. 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 primary screening of the anticancer drugs 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- 7.5) for 3- 4 days and acclimatized to laboratory conditions at about 21^0C- 26^0C

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

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 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 predetermined chronic concentration (LC_{50}/10 value of 96 hours) of 5- fluorouracil (4.078 ppm). On 10^{th}, 20^{th} and 30^{th} 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 60^0C- 70^0C till constant weight was obtained and blended into dry powder.

10 mg. of dry powder of tissue was homogenized with small amount of 10 % tricarboxylic acid (TCA) and the homogenate was diluted to 10 ml by 10 % TCA. Then it was centrifuged at 3000 rpm for 15 minutes. The supernatant removed was used for ascorbic acid estimation. Contents of Ascorbic acid of dry powders of tissues were estimated by Roe's method (1967). Optical density of color developed was read at 530 nm on a colorimeter.

1.0 ml of 10 % tricarboxylic acid similarly treated was used as a blank. Ascorbic acid contents in different tissues were calculated referring to standard graph values and it is expressed in terms of mg of ascorbic acid /100 mg of dry tissue. The L-ascorbic acid was used as a standard.

Results and Discussion

The values given in the Table indicate changes in ascorbic acid level in 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. Ascorbic acid contents in 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 ascorbic acid contents in experimental bivalves than control group. It was also observed that decrease in ascorbic acid contents 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 reduction of ascorbic acid contents in various tissues of experimental bivalves may be due to oxidative stress induced by cisplatin.

Potent anticancer drugs, cisplatin and 5-fluorouracil have serious side effects which affects the biochemical components in all exposed animal models. Although higher doses of cisplstin 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.

Table: Ascorbic acid 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	0.747 ± 0.0264	0.729 ± 0.0264	0.713 ± 0.0677
		Cisplatin (1.007 ppm)	0.608 ± 0.0308* (-18.608)	0.591 ± 0.0416* (-18.930)	0.538 ± 0.0484* (-24.544)
		5FU (4.078 ppm)	0.695 ± 0.0353* (-6.961)	0.643 ± 0.0245* (-11.797)	0.608 ± 0.0225* (-14.726)
2	Foot	Control	0.938 ± 0.0282	0.921 ± 0.0301	0.904 ± 0.0621
		Cisplatin (1.007 ppm)	0.851 ± 0.0384* (-9.275)	0.834 ± 0.0577* (-9.446)	0.780 ± 0.0634* (-13.717)
		5-FU (4.078 ppm)	0.903 ± 0.0483* (-3.731)	0.887 ± 0.0462* (-3.692)	0.851 ± 0.0395* (-5.863)
3	Gonads	Control	0.886 ± 0.0916	0.868 ± 0.0344	0.851 ± 0.0612
		Cisplatin (1.007 ppm)	0.834 ± 0.0265* (-5.869)	0.747 ± 0.0161* (-13.940)	0.661 ± 0.0326* (-22.326)
		5-FU (4.078 ppm)	0.851 ± 0.0311* (-3.950)	0.81 ± 0. 0688* (-5.991)	0.713 ± 0.0335* (-16.216)
4	Digestive glands	Control	1.095 ± 0.0750	1.077 ± 0.0284	1.060 ± 0.0162
		Cisplatin (1.007 ppm)	1.008 ± 0.0562* (-7.945)	0.938 ± 0.0183* (-12.906)	0.817 ± 0.0862* (-22.925)
		5-FU (4.078 ppm)	1.060 ± 0.0361* (-3.196)	0.973 ± 0.0974* (-9.656)	0.851 ± 0.0372* (-19.717)
5	Gills	Control	0.782 ± 0.0229	0.764 ± 0.0164	0.747 ± 0.0289
		Cisplatin (1.007 ppm)	0.696 ± 0.0421* (-10.997)	0.608 ± 0.0280* (-20.419)	0.522 ± 0.0346* (-30.120)
		5FU (4.078 ppm)	0.747 ± 0.0348* (-4.475)	0.678 ± 0.0173* (-11.256)	0.591 ± 0.0332* (-20.883)

(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: *$p<0.05$, **$p<0.01$, ***$p<0.001$, ^{NS}= Non-significant).

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., 2002 and Cetin *et al.*, 2006). The decrease in activity of the antioxidant enzymes may also be involved in oxidative stress observed in cisplatin treated rats (Koc *et al.*, 2005 and Ajith *et al.* 2007).

Vitamins are antioxidants since they prevent the toxic effects of free radicals, which can interact with various cellular molecules inhibition or alteration of functions through

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

lipid peroxidation, DNA damage and enzyme inactivation. Vitamins possess chemical properties which allow them to control the oxidation-reduction reactions of the cell and thus are frequently used in cancer therapies (Prasad, 1995). Antioxidant plays a role in the treatment of lead poisoning (Gurer *et al.*, 2001). Ascorbic acid content was depleted in various tissues and elevated in foot of bivalve *Parreysia cylindrica* after acute and chronic treatment of cypermethrin (Waykar *et al.*, 2001). Ascorbic acid can act as a hydrogen carrier, it may have essential role in metabolism of carbohydrate or protein or both. At stressful condition on exposure to toxicants ascorbic acid indicates positive role in detoxification Mahajan and Zambare, 2001). Ascorbic acid supplementation was found to recover the lead induced kidney function in rat (Sumathi and Jeyanthi, 2005). Ascorbic acid content were decreased after acute exposure to mercuric chloride and sodium arsenate while ascorbic acid treatment showed faster recovery on exposure with caffeine in fresh water bivalve, *Lamellidens corrianus* (Gulbhile, 2006).

Conclusion

Ascorbic acid contents are found to be significantly decreased in mantle, foot, gonads, digestive glands and gills of experimental bivalves, *Parreysia corrugata* as compared to control group of bivalves.

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