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Effect of Anticancer drugs Cisplatin and 5-Fluorouracil on Collagen Contents in fresh water bivalve, *Parreysia corrugata (M).*

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

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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,Parreysiacorrugata for 30 days. TheCollagencontents were estimated from different tissues of control and treated bivalves by using the method of determination of hydroxyproline in tissues as given by Woessner (1961). It was found that collagencontents were decreased in tissues with increase in period of exposure to experimental bivalves. It was also observed that the decrease in amount of collagen content in tissues was more in cisplatin treated bivalves than that of 5- fluorouracil treated bivalves.*

Introduction:

The pollutants or toxicants in environment like trace metals such as mercury; cadmium, lead etc. are known to be non-biodegradable and highly toxic to an 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 1991), due to their ability to form complexes with ligands (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 (Prestayko et al., 1979 and Rozenewig et al., 1977). Cisplatin is very effective anticancer drug widely used in the treatment of the bladder, testis, ovary and other solid tumors (Broach et al., 1987).5-fluorouracil is pyrimidine analogue and is converted in the body to the 5-fluoro-2-deoxypyrimidine monophosphate,which inhibits thymidilate synthetase and blocks the conversion of deoxyuridilic acid to deoxythymidilic acid and stops pyrimidine synthesis by inhibiting thymidilate synthetase. Thus it inhibits the DNA synthesis and blocks the cell cycle at s-phase. Therefore, 5-fluorouracil acts as a potent antitumor agent (Bhosale, 2009).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).

Collagen is one of the most important structural proteins which provide the support to the tissues. Collagen has mechanical ability to resist distending force of other tissues. The tensile strength of collagen is so high and can be compared weight for weight with that of steel. It forms important structural substance or cement support of the body which holds the tissues and organs together. Collagen provides toughness and flexibility to the bones and prevents brittleness. The brittle bone can fracture on slightest injury, weakened blood vessels could damage and rupture fast and can lead to hemorrhage, muscle weakness and pains in joints and teeth. Collagen is associated with the entire aging process. Ascorbic acid is essential factor for collagen synthesis. Ascorbic acid deficient cells as in disease scurvy, procollagen chains are not hydrolyzed to form triple helix of collagen without structural support of collagen blood vessels, skin and a muscle becomes fragile. Most of the collagen is fibrilar and made up of type I molecules. A two dimensional network of type IV collagen is found in basal lamina. Increase in stability of collagen occurs due to increased number of inter and intramolecular cross linkages (Verzer, 1961; Hall, 1976).Increased cross linking of collagen in extracellular space restricts the passage of metabolites and essential gases to concerned tissues cause physiological importance of the organ (Kohn, 1978).

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

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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^{0}C- 26^{0}C 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 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^{0}C- 70^{0}C till constant weight was obtained and blended into dry powder.

Collagen estimation was carried out by using the method of determination of hydroxyproline in tissues as given by Woessner (1961).

For collagen estimation, tissue extract obtained from 50 mg dried powder of mantle, foot, gonads, digestive glands and gills, which were taken in pyrex test tubes containing 10 ml of 6 N HCl. Test tubes were sealed by cotton ball and aluminum foil. It was hydrolyzed at 130^{0}C in autoclave. After 3 to 4 hours test tubes removed from the autoclave and hydrolysate was used as a sample.

To maintain the PH 6 to 7, 10 ml 2.5 N NaOH and 2 to 3 drops of 0.02 % methyl red indicator was added in each sample. The mixture was shaken well and appropriate quantity of 2.5 N NaOH was further added (with constant shaking while addition) till the color of sample changed from green to slight yellow indicating PH 6-7. The sample in each test tube was then taken in volumetric flask and the volume was made 50 ml by adding distilled water. This sample is then used for collagen estimation.

2.0 ml of each sample of tissue was taken in separate test tube. 1.0 ml chloramine-T reagent was added in each sample, mixed well and allowed to stand for 20 minutes at room temperature. In that mixture 1.0 ml of perchloric acid was added, mixed well and allowed to stand for next 30 minutes at room temperature. Then 1.0 ml dimethyl amino benzaldehyde was added to above mixture. It was shaken well and kept in water bath at 60^{0}C for 20 minutes. After that test tubes were taken out of water bath, cooled and O.D. was read at 557 nm on colorimeter.

The hydroxyproline was used as a standard. Hydroxyproline contents of collagen in different tissues were calculated by referring to standard graph values. The hydroxyproline contents were multiplied by a factor 7.46 to obtain the collagen content. It is expressed in mg /100 mg dry weight of tissues.

Results and Discussion:

The values given in the Table indicate changes in collagen 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. Collagen level of different tissues of Parreysia corrugata on chronic exposure to cisplatin and 5-fluorouracil 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 the table indicates percentage decrease of collagencontents in treated bivalves than those of control group. It was also observed that the decrease in collagen 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.

Nedugorac and Jacob (1993) reported that, to correlate the collagen content with myocardial mechanical parameters, the knowledge of collagen types and their distribution in normal and diseased cardiac tissue is essential. The definite changes in the collagen parameters may be of great importance to clear the mechanism of cardiac hypertrophy, heart failure or pathogenesis of other cardiac diseases. DNA content is drastically affected due to the stress condition caused by toxic exposure to heavy metals. It occurs only due to certain physiological burden or period of exposure.Zeolite in catfish, Heteropneustes fossilis reduced nucleic acid and protein contents in exposed fish (James and Sampath, 2000). The complex mechanism of collagen synthesis involves several enzymes, so that congenital deficiency of any of the enzyme can lead to some disorder of its pathway. It means there may be wide variety of clinical symptoms associated with such disorders; there may be fragile bones, fractures from little trauma, delicate blood vessels, dental defects, readily dislocating joints, a bent or twisted spine, hyper elastic skin and poor wound healing.

The alteration in collagen phenotypes may be responsible for malfunction in hypertensive heart disease (Thiedmann et al. 1983).Michael (1996) reported that in kidney s, the formation of a relatively protein free ultra filtrate by the glomerulus involves a complex interplay structural, biochemical and homodynamic factors. Kidney has its own pattern of collagen type distribution.Ohyama, et al., (1990) reported that any imbalance in the extracellular matrix or alteration in the metabolism of collagen in a pathological condition such as glomerulosis leads to significantly reduced glomerular function.

Table: Collagen contents in different tissues of Parreysia corrugata on exposure tochronicdose of Cisplatin and 5-Fluorouracil.

Sr. No.	Tissue	Exposure to	10 Days	20 Days	30 Days
1	Mantle	Control	2.72 ± 0.0191	2.66 ± 0.0206	2.56 ± 0.0330
		Cisplatin (1.007 ppm)	2.41 ± 0.0263*** (-11.397)	2.36 ± 0.0727* (-11.278)	2.01 ± 0.182* (-21.484)
		5-FU (4.078 ppm)	2.56 ± 0.0299* (-5.882)	2.46 ± 0.0816* (-7.519)	2.18 ± 0.0387** (-14.844)
2	Foot	Control	3.15 ± 0.0479	3.10 ± 0.0984	3.00 ± 0.360
		Cisplatin (1.007 ppm)	2.66 ± 0.0377** (-15.556)	2.58 ± 0.136* (-16.774)	2.26 ± 0.0556* (-24.667)
		5-FU (4.078 ppm)	2.93 ± 0.0818* (-6.984)	2.86 ± 0.197* (-7.742)	2.51 ± 0.0763* (-16.333)

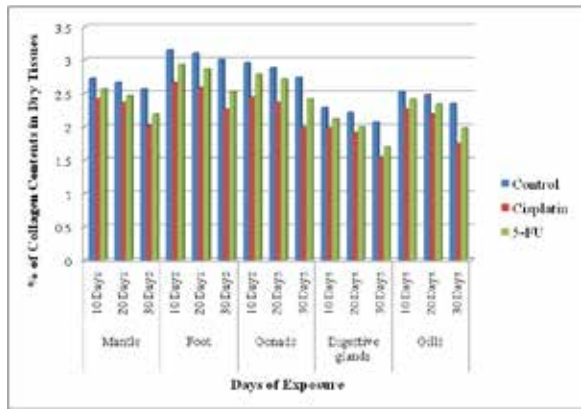
Volume : 5 | Issue : 1 | Jan 2015 | ISSN - 2249-555X

3	Gonads	Control	2.95 ± 0.0881	2.88 ± 0.168	2.73 ± 0.217
		Cisplatin (1.007 ppm)	2.44 ± 0.471* (-17.288)	2.36 ± 0.0525* (-18.055)	1.99 ± 0.116* (-27.106)
		5-FU (4.078 ppm)	2.78 ± 0.0660* (-5.763)	2.71 ± 0.179* (-5.903)	2.41 ± 0.0953* (-11.722)
4	Digestive glands	Control	2.28 ± 0.215	2.21 ± 0.125	2.06 ± 0.0173
		Cisplatin (1.007 ppm)	1.98 ± 0.207* (-13.158)	1.91 ± 0.0957* (-13.575)	1.54 ± 0.146* (-25.243)
		5-FU (4.078 ppm)	2.11 ± 0.0665* (-7.456)	1.99 ± 0.115* (-9.995)	1.69 ± 0.0525* (-17.961)
5	Gills	Control	2.51 ± 0.213	2.48 ± 0.0526	2.34 ± 0.0129
		Cisplatin (1.007 ppm)	2.26 ± 0.0967* (-9.960)	2.18 ± 0.0432*** (-12.097)	1.74 ± 0.0287* (-25.641)
		5-FU (4.078 ppm)	2.41 ± 0.167* (-3.984)	2.33 ± 0.0252* (-6.048)	1.98 ± 0.331* (-15.385)

(Values are expressed in terms of 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).

The pathway of increased collagen degradation has been associated with accelerated activation of collagenase (Weber et al., 1988 and Reddy et al., 1993) and lysosomal degradation enzymes due to the heavy metal stress (Takahashi et al., 1990).Rajashree and Puvandkrishnan (2000) found that total collagen content in heart and kidney decreased on eight days of dexamethasone treatment in rat. The decrease in I: III ratio of collagen was found in kidney Rajashree and Puvandkrishnan (2000).

Figure: Collagen contents in different tissues of Parreysia corrugata after Chronic Exposure to Cisplatin and 5-Fluorouracil.



Conclusion:

Collagen contents are found to be significantly decreased in mantle, foot, gonads, digestive glands and gills of experimental bivalves, Parreysia corrugataon chronic exposure to cisplatin and 5-fluorouracil as compared to control group of bivalves.

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