INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

CHELATING PROPERTIES OF *CARDIOSPERMUM HALICACABUM* AGAINST THE TOXIC EFFECT OF CYPERMETHRIN IN THE QUANTIFICATION OF NUCLEIC ACIDS IN THE FRESH WATER FISH *CIRRHINUS MRIGALA* (HAMILTON)

Vasantharaja C, K.Pugazhendy*, M.Meenambal, S. Prabakaran and Nisar-Ahmad-Dar

Department of zoology, Faculty of science, Annamalai University, Annamalai nagar, Tamil Nadu, India

Article Received on: 18/11/12 Revised on: 11/12/12 Approved for publication: 30/12/12

*Email: pugalendy@rediffmail.com

ABSTRACT

Among the pesticides the synthetic pyrethroids are commonly used because of their rapid biodegradability and non-persistent nature. The cypermethrin has a strong pescicidal activity in fresh water fish *C. mrigala* for all exposure periods (24h to 120h) The acute toxicity value was found to be 150 µg/l and 1/5 as used for LC_0 (30µg/l). Where observed on DNA and RNA content in selected tissues like gill, liver and kidney of fresh water fish. An overall decrease in nucleic acid was noted in (group 2) which is statistically significant. This indicates an evidence of inhibition of these nucleic acids in the selected organs by the toxicant problem *Cardiospermum halicacabum* exposed (group 3). Shows recovered by *Cardiospermum halicacabum*, Hence the pesticide intoxication has made a disturbance in normal functions of cells.

Key words: Cypermethrin, *Cirrhinus mrigala, Cardiospermum halicacabum,* DNA and RNA

INTRODUCTION

The control of pest involves the heavy use of synthetic pesticides, but the wide-spread use of these substances has led to serious problem including toxic residue on grass and toxicity to non target organisms such as mammals, birds and fishes. These compounds also interfere with many vital physiological functions and constituently alter the levels of various biochemical constituents in fishes [1].

Application of pesticides has increased manifolds in the modern world to get immediate relief from various indoor, outdoor, and agricultural pests, ignoring devastating effects of these pesticides on different non-target flora and fauna. These pesticides ultimately reach the aquatic systems through different pathways, affecting various aquatic organisms. Besides direct deposition or drift, these compounds may reach aquatic habitats via runoff, depending upon precipitation, soil conditions, and slope of the catchment area [2]. Pyrethroids are now commonly used as broad-spectrum insecticides having neurotoxic properties [3].

The cyano pyrethroids (cypermethrin and k-cyhalothrin) are known to affect the nervous system of organisms, exhibiting complex poisoning symptoms ultimately leading to death. As with target pests, nontarget organisms, including aquatic invertebrates and fish, have been reported to be extremely sensitive to the insecticides [4, 5].

Cellular RNA content is correlated with the rate of protein synthesis. DNA content, which remains relatively constant in somatic tissues, may be used as an index of cell number6. The RNA and DNA ratio reflects the protein synthesizing capability of larval fish and has been used for estimating recent in situ protein growth[6,7,8,9].

According to the World Health Organization[10] "a medicinal plant" is any plant which in one or more of its organ contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. The therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant used for preparing medicines (for example: leaves, flowers, seeds, roots, barks, stems)[11].

Critical review of the literature in this context suggests that research work regarding biochemical evaluation of the effects of cypermethrin compounds on the levels of nucleic acids (DNA and RNA) in non target fish species has not received much attention. Therefore, in an attempt to establish the correlation between the biochemical changes caused by cypermethrin exposure with the variation in these key regulatory elements (DNA, RNA) of the cell. This study was undertaken to investigate the impact of subacute concentrations of these cypermethrin on the status of DNA and RNA in different tissues of *C. mrigala*, and also observe the chelating mechanism of *C. halicacabum* exposed for 120hrs.

MATERIALS AND METHODS

The fish *Cirrhinus mrigala* of size [14] to 16 cm and 50 to 70g weight were brought from a local fish farm in Pinnaloor, at Navarathna form. Fish collected and acclimatized at 28°C in the large sized aquarium tank disinfected with potassium permanganate and washed thoroughly prior to conduction of fish to prevent the fungal disease for acclimatization in the laboratory condition for 15 days. During laboratory condition fishes were feed with artificial feed, water was renewed on every day to maintain water quality. The LC_{50} concentration of cypermethrin was noted at 120 hrs. Fish organs gill liver and kidney were collected by dissection the animal and stored at -20^{0}C for nucleic acids studies. Fishes were exposed in 4 groups.

Group-1 fish exposed to tap water (control)

Group- 2 fish exposed to cypermethrin

Group-3 Fish exposed to cypermethrin along with *Cardiospermum halicacabum*

Group-4 Fish exposed to *Cardiospermum halicacabum* alone

Plant preparation

Healthy disease free leaves of *Cardiospermum halicacabum* were collected from Villupuram district Nallavur Village in January-2011 and plant was identified. The leaves were washed in running tap water for 10 minutes leafs were dried, aerial parts (1kg) of *Cardiospermum halicacabum* were macerated thrice at room temperature and prepared in powdered condition and equal amount of rice bran mixed

well and small amount water added and prepared small pellet as feed.

Organs nucleic acid assay

DNA and RNA content were determined in the tissues by the method of [12].

Statistical analysis

The data obtained in the present work were expressed as means ± SE, percentage changes and were statistically analyzed using student t-test[13] , to compare means of treated data against their control ones and the result were considered significant at (P <0.05), (P<0.01) level.

RESULT AND DISCUSSION

The variations of deoxyribo nucleic acid (DNA) and Ribonucleic acid (RNA) observed in the tissue of gill, liver and kidney tissue of *Cirrhinus mrigala* during sub lethal concentration of cypermethrin for 24, 48, 72, 96 and 120 hours of exposure periods. The DNA activity significantly decreased in compared to control group-1 in all tissue during the toxic exposure periods. The fish was exposed to group-3 the DNA and RNA content was regained when compared to group-2. While in the fish exposed to group-4 when compared with group-1 the slightly increased. The recorded DNA and RNA contents were statistically significant at 5% and 1% levels (Table-1, 2).

Impairment of nucleic acid metabolism caused the degradation of cells, resulting in the reduction in the DNA content. Inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. The regulatory role of nucleic acid metabolism as observed in the different animals when treated with the different pesticides reported earlier[14]. Pesticide appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the organophosphate (OP) compounds may attack many enzymes responsible for normal metabolic pathway [15].

RNA plays significant role in protein synthesis hence depletion in RNA contents also results in depletion in protein level. Hence, there is decrease in RNA level thus reducing protein synthesis [16]. [17] fish exposed to Dimethoate (organophosphate) exhibited a decrease in nucleic acid (DNA and RNA) content. The reason for decreased nucleic acids levels in liver under the influence of carbosulfan treatment in mice might cause genotoxic action by decreased mitotic index and disturbed cell division.

Table-1 variations of Deoxyribonucleic acid (U/min/mg protein) in the freshwater fish *C. mrigala* exposed to cypermethrin and *C. halicacabum* exposed to 120 hours

Tissue		Hours of experiment				
		24	48	72	96	120
Gill	Group-1	35.087 ± 0.674	35.091 ± 0.986	35.093 ± 0.850	35.095 ± 0.891	35.094 ± 0.770
	Group-2	32.123* ± 0.706	31.228* ± 0.803	30.069** ± 0.873	29.089** ± 0.755	28.035** ± 0.923
	%COC	% -8.45	% -11.01	% -14.32	% -17.11	% -20.11
	Group-3	33.480NS ± 0.870	33.076NS ± 0.608	32.176* ± 0.712	31.901* ± 0.706	31.867* ± 0.900
	% COC	% -4.56	% -5.74	% -8.31	% -9.10	% -9.19
	% COT	% 4.22	% 5.92	% 7.01	% 9.67	% 13.67
	Group-4	35.096NS ± 0.650	35.105NS ± 0.812	35.116NS ± 0.722	35.124NS ± 0.974	35.130NS ± 0.712
	% COC	% 0.02	% 0.04	% 0.06	% 0.08	% 0.10
Liver	Group-1	41.429 ± 0.693	41.434 ± 0.570	41.435 ± 0.734	41.437 ± 0.877	41.435 ± 0.804
	Group-2	38.606* ± 0.733	36.129** ± 0.644	33.876** ± 0.853	32.612** ± 0.944	31.424** ± 0.816
	%COC	% -6.81	% -12.80	% -18.24	% -21.30	% -24.16
	Group-3	39.876NS ± 0.518	39.180NS ± 0.670	38.581* ± 0.661	38.098* ± 0.580	37.550* ± 0.674
	% COC	% -3.75	% -5.44	% -6.89	% -8.06	% -9.38
	% COT	% 3.29	% 8.44	% 13.89	% 16.82	% 19.49
	Group-4	41.440NS ± 0.818	41.453NS ± 0.714	41.460NS ± 0.463	41.469NS ± 0.644	41.475NS ± 0.720
	% COC	% 0.03	% 0.05	% 0.06	% 0.08	% 0.10
Kidney	Group-1	38.118 ± 0.544	38.122 ± 0.969	38.125 ± 0.870	38.127 ± 0.644	38.126 ± 0.518
	Group-2	35.786* ± 0.670	33.670* ± 0.580	32.013** ± 0.774	30.118** ± 0.818	29.511** ± 0.594
	%COC	% -6.12	% -11.68	% -16.03	% -21.01	% -22.60
	Group-3	36.913NS ± 0.604	36.077NS ± 0.669	35.320* ± 0.574	34.816* ± 0.811	33.905** ± 0.614
	% COC	% -3.16	% -5.36	% -7.36	% -8.64	% -11.07
	% COT	% 3.15	% 7.15	% 10.33	% 15.60	% 14.89
	Group-4	38.129NS ± 0.870	38.143NS ± 0.691	38.155NS ± 0.588	38.167NS ± 0.519	38.172NS ± 0.871
	% COC	% 0.03	% 0.05	% 0.08	% 0.10	% 0.12

Values are mean ± SE of six replicates, percentage changes and student t-test. Significant at *P<0.05; ** P<0.01 levels; % COC- change over control; % COT- change over treated

Table-2 variations of Ribonucleic acid (U/min/mg protein) in the freshwater fish *C. mrigala* exposed to cypermethrin and *C. malicacabum* exposed to 120 hours

Tissue		Hours of experiment				
		24	**48**	**72**	**96**	**120**
Gill	Group-1	83.776 ± 1.0[18]	83.781 ± 1.191	83.784 ± 1.026	83.786 ± 1.177	83.787 ± 0.986
	Group-2	80.107[NS] ± 1.[18]0	78.627* ± 1.241	76.0[13]** ± 1.038	74.086** ± 1.106	73.009** ± 1.270
	%COC	% -4.38	% -6.15	% -9.27	% -11.58	% -12.86
	Group-3	81.480[NS] ± 1.008	80.986[NS] ± 1.108	80.012* ± 1.116	79.127* ± 1.[18]0	78.775* ± 1.284
	% COC	% -2.74	% -3.37	% -4.450	% -5.56	% -5.98
	% COT	% 1.71	% 3.00	% 5.26	% 6.80	% 9.27
	Group-4	83.789[NS] ± 1.[18]3	83.799[NS] ± 1.297	83.807[NS] ± 1.080	83.817[NS] ± 1.1[18]	83.820[NS] ± 1.096
	% COC	% 0.01	% 0.02	% 0.03	% 0.04	% 0.04
Liver	Group-1	91.084 ± 1.080	91.089 ± 1.176	91.093 ± 1.1[18]	91.095 ± 1.047	91.096 ± 1.[18]3
	Group-2	88.071[NS] ± 1.[14]3	85.060* ± 1.066	83.271** ± 1.168	81.374** ± 1.0[14]	79.096** ± 1.270
	%COC	% -3.31	% -6.62	% -8.57	% -10.67	% -[13].17
	Group-3	89.886[NS] ± 1.127	88.726[NS] ± 1.2[14]	87.155* ± 1.009	86.480* ± 1.150	85.378* ± 1.078
	% COC	% -1.31	% -2.59	% -4.34	% -5.07	% -6.28
	% COT	% 2.06	% 4.31	% 4.66	% 6.27	% 7.94
	Group-4	91.099[NS] ± 1.170	91.112[NS] ± 1.096	91.121[NS] ± 1.280	91.[13]5[NS] ± 1.1[18]	91.[13]8[NS] ± 1.077
	% COC	% 0.02	% 0.02	% 0.03	% 0.04	% 0.05
Kidney	Group-1	86.2[18] ± 1.070	86.221 ± 1.[13]4	86.230 ± 1.004	86.236 ± 1.071	86.240 ± 1.096
	Group-2	84.020[NS] ± 1.[13]0	81.2[18]* ± 1.280	79.240** ± 1.078	76.1[18]** ± 1.196	74.086** ± 1.270
	%COC	% -2.55	% -5.80	% -8.11	% -11.73	% -[14].09
	Group-3	84.988[NS] ± 1.273	84.0[13][NS] ± 1.176	83.693* ± 1.083	82.4[13]* ± 1.007	82.005* ± 1.178
	% COC	% -1.43	% -2.56	% -2.94	% -4.43	% -4.91
	% COT	% 1.15	% 3.44	% 5.62	% 8.53	% 10.69
	Group-4	86.229[NS] ± 1.207	86.240[NS] ± 1.270	86.255[NS] ± 1.126	86.269[NS] ± 1.1[18]	86.275[NS] ± 1.083
	% COC	% 0.01	% 0.02	% 0.03	% 0.04	% 0.04

Values are mean ± SE of six replicates, percentage changes and student t-test. Significant at *P<0.05; ** P<0.01 levels; % COC- change over control; % COT- change over treated

The quantity of protein depends on the rate of protein synthesis or its degradation. It also affected due to impaired incorporation of amino acids into polypeptide chains [18]. The synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis transcription level, thus may affect the protein level. In this study, a significant decline in RNA level exposed freshwater fish was observed. The decrease in the RNA concentration may also have been a cause of protein depletion. Alternatively, the increase in protease activity may be the cause of increased protein degradation.

Cypermethrin was also significantly decreased the level of nucleic acids in the various tissues of the fish *C. fasciatus,* reports are available on the reduction in DNA and RNA level on exposure to different pesticides[19]. Many physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and wide range of anti-infective action have been assigned to tannins[20]. While in the fish exposed to group 3 (*Cardiospermum halicacabum* plant feed group). Shows regained from reduction nucleic acid content. The plant *C. halicacabum* have rich amount of alkaloid, flavonoids and sponins.

Alkaloid production is a characteristic of all plant materials. They exhibit marked physiological activity when administered to animals [20]. Furthermore, alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs [21], [22]. In terms of anti-cancer activity, they inhibit the initiation, promotion and progression of tumors [23], [24]. In recent years, plant flavonoids have attracted attention as potentially important dietary cancer chemo-protective agents[26]. Some isoflavones act as allelochemicals widely used in insecticides [26].

Saponins have been shown to possess both beneficial (lowering cholesterol) and deleterious (cytotoxic and permeabilization of intestinal epithelium) properties and to exhibit structure dependent biological activity. In medicine, it is used to some extent as an expectorant and an emulsifying agent [21].

CONCLUSION

In the present investigation it can be concluded that under exposure to pesticides cypermethrin the DNA and RNA content decreased in gill, liver and kidney tissues of the fish *C. mrigala* leading to recovered effect by *Cardiospermum halicacabum* plant.

REFERENCES

1. Sharma DK and Ansari BA. Effect of Deltamethrin and a neem-based pesticide Achook on some biochemical parameters in tissues liver, ovary and muscle of zebrafish, *Danio rerio* (Cyprinidae). Res J Chem Sci2011:1: 125-134.
2. Liess M, Schulz R, Liess MHD, Rother B and Kreuzig R. Determination of insecticide contamination in agricultural headwater streams. Water Res 1999:33:239–247.
3. Salibian A. Effects of Deltamethrin on the South American toad, Bufo arenarum, tadpoles. Bull Environ Contam Toxicol 1992:48:616–621.
4. Philip GH and Rajasree BH. Action of cypermethrin on tissue transamination during nitrogen metabolism in *Cyprinus carpio*. Ecotoxicol Environ Saf. 1996:34:174–179.
5. KoprucuSS, Koprucu K and Ural MS. Acute toxicity of the synthetic pyrethroid Deltamethrin to fingerling European catfish, Silurus glanis, L. Bull Environ Contam Toxicol 2006: 76:59–65..
6. Bulow FJ. RNA-DNA ratios as indicators of growth in fish: a review. *In* R. C. Summerfelt and G. E. Hall eds.l, Age and growth in fish1987: p. 45-64. Iowa State Univ. Press, Ames, Iowa.
7. Robinson SMC and Ware DM. On to genic development of growth rates in larval Pacific herring, *Clupea harengus pallasi,* measured with RNA DNA ratios in the Strait of Georgia, British Columbia. Can. J. Fish. Aquat. Sci 1988:45: 1422-1429.
8. Hovenkamp F. Growth differences in larval plaice *Pleuronectes platessa* in the southern bight of the North Sea as indicated by otolith increments and RNA/DNA ratios. Mar. Ecol. Prog. Ser1990: 58:205-215.
9. Hovenkamp F and Witte IJ. Growth, otolith growth, and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987 to 1989. Mar. Ecol. Prog. Ser1991: 70:105-116.
10. Anonymous, Medicinal, culinary and Aromatic Plants in the near East-Medic… http://www.faO.Org/dorep/x5402e16.htm (cited 4 August, 2007).
11. Schneider WC. Phosphorous compounds in animal tissues 1. Extraction and estimation of deoxypentose nucleic acid. J. Biol Chem1957:161:293-298.
12. WHO. Resolution –Promotion and Development of Training and Research in Traditional Medicine. 1977. WHO document No: 30-49.
13. Milton TS and Tsokos JO. Statistical methods in the biological and health science. McGraw – will. Internet Book comp., 1983.pp381-405.
14. Das BK and Mukherjee SC. Comp. Biochem and Physiol. (Part C) 2003:134: 109–121.
15. Tripathi G and Verma p. Comp. Biochem. Physiol 2004:138(1): 75–79.
16. Maruthanayagam C and Sharmila G. Nature Env. Polln. Techn2004: 3(4): 491– 494
17. Tripathi P. K *et al.*, Asian fisheries Sci 2003: 16:349–359.
18. Singh N.N, DasVK and Singh S. Effect of aldrin on carbohydrate protein and ionic metabolism of a freshwater catfish *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol 1996:57(2): 204-210.
19. Mahendru VK. Studies on Pharmacology of molluscicides on the gastropod *Lymnaea acuminata*. Ph.D. Thesis1981: Department of Zoology, Gorakhpur University, Gorakhpur, (U.P.) India.
20. Okwu DE and Okwu ME. Chemical composition of *Spondias mombin* Linn. Plant parts. J. Sustain. Agric Environ 2004: 6 (2): 140-147.
21. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of plant Analysis. Chapman and Hall Ltd, London1973:279.
22. Okwu DE, Phytochemicals, Vitamins and Mineral contents of two Nigeria Medicinal plants. Int. J.Mol. Med. Adv. Sci2005:1(4): 375-381.
23. Urquiaga I and Leighton F. Plant polyphenol, antioxidants and Oxidative stress. Biol. Res2000:33:159-165.
24. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *J. Sustain. Agric. Environ2004:* 6: 30-34.
25. Elangevan V, Sekar N and Godndasamy S. Chemopreventive Potential of Dietary Bioflavonoids against 20- Methylchofanthrene induced tumoringenesis. Cancer Lett.1994. *87*: 102-113.
26. Kandaswami CE, Perkins DS, Solonink G, Arzewiecki E., Anti-preventative effects of citrus flavonoids on human squamous cell carcinoma *in vitro.* Cancer lett 1994. 56:147-152.

Source of support: Nil, Conflict of interest: None Declared