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TOXIC IMPACT OF λ- CYHALOTHRIN ON DNA AND RNA CONTENTS OF THE FISH *CTENOPHARYNGODON IDELLA*

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Keywords:

λ-cyhalothrin (5% EC), *Ctenopharygodon idella*, Sublethal concentrations, DNA and RNA

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ABSTRACT: The synthetic pyrethroids used in agriculture also absorb rapidly *via* different routes and slowly accumulate into various vital tissues and cause adverse effects on the health status of animals. It leads to produce a number of biochemical changes at the level of nucleic acids. The freshwater fish *Ctenopharyn godonidella* was exposed to sublethal concentrations (1/10th of LC50 value) of λ- cyhalothrin 5% EC for a period of 1, 4, 8 and 12 days to study the toxic impacts on DNA and RNA contents in various organs such as gill, muscle liver-kidney, and brain. In control fish, DNA content was in the order of Muscle (35.78)> Liver (34.17) > Kidney (28.74) > Brain (25.60) > Gill (18.71) and RNA was in the order of Kidney (37.15) > Muscle (36.45) > Liver (32.68) > Brain (28.15) > Gill (22.56). In the present study under sublethal concentrations of λ- cyhalothrin on day 12the percent depletion of DNA content in the test tissues were in the order of: Brain (21.43) > Gill (16.36) > Muscle (32.18) > Kidney (26.24) > Liver (31.74) and RNA content in the order of Liver (23.33)> Gill (17.54) > Brain (22.41) > Muscle (31.18) > Kidney (32.56). In the present study, results reveal that λ- cyhalothrin caused variability in the nucleic acids in different tissues and are dose-dependent. Inhibition of DNA synthesis thus might affect both proteins and amino acid levels by decreasing the level of RNA in protein machinery.

INTRODUCTION: Pesticides are known to exert specific effects in cellular processes and key proteins like DNA and RNA in regulating general metabolism, cell growth, differentiation and survival. The chronic duration of exposure to most contaminants might add another level of complexity and be considered in risk evaluation. Influence of pesticides on non-target organisms directed to minimize or eliminate the production of specific pesticides and should decrease contamination of food and other goods by pesticides using modern technologies.

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The improper management of pesticides in agricultural crops could result in contamination of water bodies [1]. Synthetic pyrethroid pesticides are frequently used in agricultural applications, residential areas and commercial facilities. They have greater photostability, enhanced insecticidal activity, and relatively low toxicity when compared to organochlorine and organophosphate pesticides.

The unrestricted heavy use of synthetic chemical pesticides results in deleterious effects odour of water, taste, lethal effect on various non-target organisms in the aquatic environment and direct or indirect effect to users [2, 3, 4]. The toxicant λ-cyhalothrin is highly toxic to fishes as it is strongly absorbed by the gills even at a very low concentration in water due to its high lipophilicity. The lipophilic nature of water-soluble pesticides increased their ability to enter the plasma membrane and come in contact with the tissues of

the aquatic organism5. Hence this study is aimed to find out the biochemical changes like DNA and RNA activity levels were observed in *Ctenopharyngodon idella* exposed to sublethal concentrations of λ-cyhalothrin for a period of [1, 4,] 8 and 12 days.

MATERIALS AND METHODS: The freshwater fish of *Ctenopharyn godonidella* were collected from ponds in Kuchipudi, Guntur District, brought to the laboratory, then acclimatized to the laboratory conditions in large plastic tanks with unchlorinated groundwater for two weeks at a room temperature of 28 + 20 ℃ prior to experimentation. LC_{50} for 96 h was found out by using probit method [6]. For biochemical studies, fishes were reared in sublethal concentration for a period of 1, 4, 8, and 12 days. The nucleic acids DNA and RNA were estimated by method [7].

RESULTS AND DISCUSSION: The calculated values for *Ctenopharyn godonidella* exposed to sublethal concentrations of λ- cyhalothrin for 1, 4, 8, and 12 days were given in **Table 1** & **2.** The nucleic acids' DNA and RNA contents were depleted in all the tissues of test fish *Ctenopharyngodon idella* compared with controls. In the control fish, the DNA content was in the order of Muscle > Liver > Kidney > Brain > Gill. Under sublethal exposure to λ-cyhalothrin for 1 day, maximum percent of depletion was inbrain (3.67%)followed by gill (-2.46%), muscle (2.35%), kidney (-2.05%) and minimum depletion in liver (1.43%); for day 4 maximum depletion in gill (6.62%) followed by muscle (-6.18%), kidney (3.48%), brain (-3.48%) and minimum depletion in liver (-2.9%); for day 8 maximum depletion in brain (-10.91%) followed by gill (-8.82%), muscle (-8.13%), kidney (-6.64%) and minimum depletion in liver (-4.68%) and for day 12 the maximum percentage of depletion in brain (-16.29%) followed by gill (-12.56%), muscle (-10.06%), kidney (-8.69%) and minimum depletion was in liver (-7.11%) were observed in test fish *Ctenopharyngodon idella*. In the control fish Ctenopharyngodo nidella the RNA content was in the order of Kidney >Muscle > Liver > Brain > Gill. Under sublethal exposure toλ-cyhalothrin for 1 day, observed maximum percentage of depletion in liver (-7.1%) followed by gill (-5.5%), muscle (1.89%), kidney (-1.32%) and minimum depletion was observed in brain (-1.17%); for day 4 maximum depletion in gill (-12.9%) followed by liver (-10.22%), brain (-5.75%), muscle (-5.19%) and minimum depletion in kidney (-4.55%); for day 8 maximum depletion in gill (-17.82%) followed by liver (-15.42%), brain (-9.2%), muscle (-7.93%) and minimum depletion in kidney (-6.27%) and for day 12 the maximum percentage of depletion in liver (-28.61%) followed by gill (-22.25%), brain (20.39%), muscle (-14.46%) and minimum depletion in kidney (-12.36%) were observed in test fish *Ctenopharyn godonidella*.

In the present study, DNA and RNA contents showed a marked decrease in the exposure periods, and the results indicated that almost all the tissues of the brain, muscle, gill, kidney and liver were affected by this synthetic pyrethroid at sublethal exposures.

In the present study, the rapid histolysis in tissues because of λ-cyhalothrin treatment to the fish *Ctenopharyn godonidella*, impairment of nucleic acid metabolism, and the degradation of cells, results in the reduction of DNA content.

TABLE 1: CHANGE IN THE SPECIFIC ACTIVITY LEVELS OF DNA (MG/GM WET WEIGHT OF THE TISSUE) AND % CHANGE OVER THE CONTROL IN DIFFERENT TISSUES OF *CTENOPHARYNGODON IDELLA* ON EXPOSURE TO SUBLETHAL CONCENTRATIONS OF Λ-CYHALOTHRIN (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sub lethal	% change	Sub lethal	% change	Sub lethal	% change	Sub Lethal	% Change
Gill	18.71±0.07	18.25±0.10	-2.46	17.47±0.02	-6.62	17.06±0.01	-8.82	16.36±0.01	-12.56
Muscle	35.78±0.08	34.94±0.06	-2.35	33.57±0.01	-6.18	32.87±0.01	-8.13	32.18±0.01	-10.06
Liver	34.17±0.02	33.68±0.07	-1.43	33.18±0.01	-2.9	32.57±0.01	-4.68	31.74±0.03	-7.11
Kidney	28.74±0.05	28.15±0.03	-2.05	27.74±0.04	-3.48	26.83±0.03	-6.64	26.24±0.06	-8.69
Brain	25.60±0.05	24.66±0.07	-3.67	23.19±0.04	-3.48	22.81±0.05	-10.91	21.43±0.02	-16.29

Results are the mean values of five observations, and the Standard Deviation is indicated as ± and figures in % change over control and sub-lethal, respectively. Values are significant at $P<0.05$

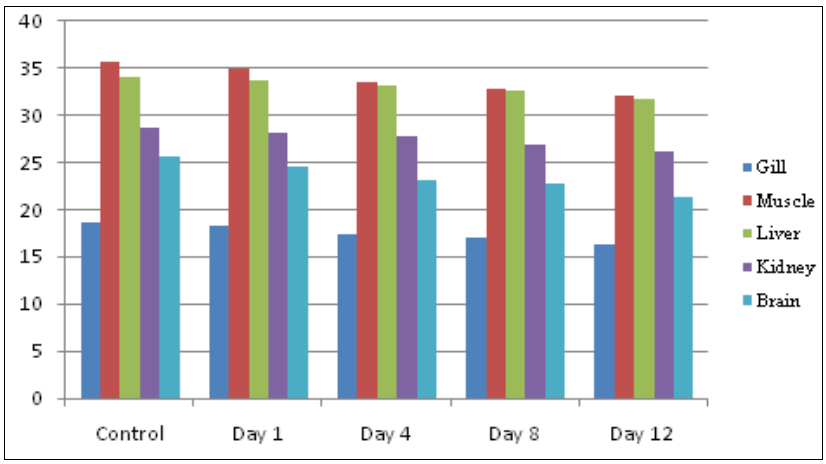

FIG. 1: CHANGE IN THE DNA (MG/GM WET WEIGHT OF THE TISSUE) AND PERCENT CHANGE OVER THE CONTROL IN DIFFERENT TISSUES OF THE FRESHWATER FISH, *CTENOPHARYNGODON IDELLA* ON EXPOSURE TO SUBLETHAL CONCENTRATIONS OF Λ-CYHALOTHRIN (5% EC)

TABLE 2: CHANGE IN THE SPECIFIC ACTIVITY LEVELS OF RNA (MG/GM WET WEIGHT OF THE TISSUE) AND % CHANGE OVER THE CONTROL IN DIFFERENT TISSUES OF *CTENOPHARYNGODON IDELLA* ON EXPOSURE TO SUBLETHAL CONCENTRATIONS OF Λ-CYHALOTHRIN (5% EC)

Tissue	Control	Exposure period (days)							
		1		4		8		12	
		Sub lethal	% Change	Sub lethal	% Change	Sub lethal	% Change	Sub Lethal	% Change
Gill	22.56±0.20	21.32±0.05	-5.5	19.65±0.04	-12.9	18.54±0.01	-17.82	17.54±0.02	-22.25
Muscle	36.45±0.19	35.76±0.03	-1.89	34.56±0.17	-5.19	33.56±0.29	-7.93	31.18±0.48	-14.46
Liver	32.68±0.04	30.36±0.23	-7.1	29.34±0.04	-10.22	27.64±0.04	-15.42	23.33±0.10	-28.61
Kidney	37.15±0.03	36.6±0.02	-1.32	35.46±0.19	-4.55	34.82±0.03	-6.27	32.56±0.19	-12.36
Brain	28.15±0.02	27.82±0.09	-1.17	26.53±0.01	-5.75	25.56±0.17	-9.2	22.41±0.17	-20.39

Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant at $p<0.05$.

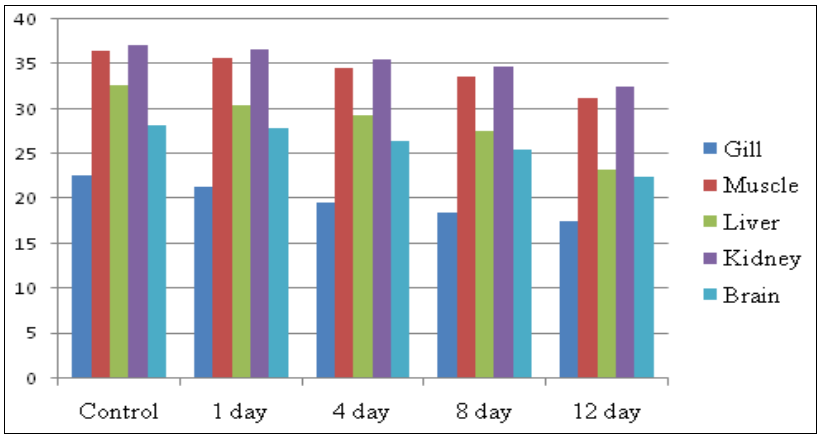

FIG. 2: CHANGE IN THE RNA (MG/GM WET WEIGHT OF THE TISSUE) AND PERCENT CHANGE OVER THE CONTROL IN DIFFERENT TISSUES OF THE FRESHWATER FISH, CTENOPHARYNGODONIDELLA ON EXPOSURE TO SUBLETHAL CONCENTRATIONS OF Λ-CYHALOTHRIN (5% EC)

Inhibition of DNA changes might affect both protein and amino acid levels by decreasing the level of RNA in protein synthesis machinery. λ-cyhalothrinas a potential inhibitor of DNA synthesis, the result might be the reduction in the RNA level because of toxicity, and many enzymes are also responsible for the normal metabolic pathway. The physicochemical interaction of the pesticides with the cellular DNA produces a variety of primary lesions such as single-strand breaks, double-strand breaks, DNA protein cross-link and damage to purine and pyrimidine bases [8]. The intactness of the DNA is an important part of the normal cellular process.

The change in DNA andRNA ratio results in eventual loss of cell structure, proliferation and formation of new tissue and tissue degradation with a total loss of cellular control mechanism [9, 10].

Present study ribonucleotidase reductase, an enzyme required for the deoxyribonucleotide synthesis, might be inactivated by the free radicals generated due to exposure to the toxic compound, which might have led to the decreased levels of deoxyribonucleotide, leading to a decline in the DNA content. Oxidative damage to the nuclear material by reactive oxygen species could also have led to the decreased nucleic acid synthesis. A direct mechanism for damage involves the reaction of hydroxyl radicals with DNA as a result of oxidative stress making DNA a suitable target for oxidative damage. Indirect mechanism of DNA damage involves Reactive Oxygen Species (ROS) mediated triggering of a series of metabolic events within the cell that might lead to inhibition or activation of a number of enzymes that replicate or repair DNA might predispose DNA to damage.

DNA and RNA dynamics during conditions of stress correspond to degenerative changes in the tissues leading to decreased protein synthesis and cellular proliferation activity.The energy diverted for maintenance of metabolism during stress by the breakdown and utilization of stored glycogen, depletion of protein and lipid. Variations in DNA and RNA contents in different tissues of treated with different types of toxicant [11]. Decreased DNA-RNA levels were observed in fish Colisafasciatus exposed to cypermethrin at different concentrations [12]. DNA and RNA content were decreased in liver, brain, and gill tissues of Channa punctatus treated with alphamethrin, due to inhibitory action of synthetic pyrethroid alphamethrin on DNA synthesis machinery or increased degradation [13].

Nucleic acid a protein content is regarded as important biomarkers of the metabolic potential of cells, as these play an important role in regulating the different activities of cells. Their ratios also provide significant information about the way in which, or the mechanism by which, these contents regulate the multifaceted activities of cells. In the present work, *Ctenopharyngodon idella* exposed to λ-cyhalothrin DNA contents were decreased in all the tissues in response to cypermethrin treatment. However, the degree of elevation was tissue-specific, and enhancement in the DNA level might be due to activation of some dominant regulating factors or increase in activity of the essential factors controlling DNA synthesis. The slight increase of DNA in gills following pyrethroid treatment might be attributed to the hypertrophic nature of chloride cells in response to the toxicant administration.The enlargement of nuclei in chloride-secreting cells in Channastriatusexposed to Metasystox corroborates the absorption or degradation of either of these pesticides or their metabolites in different tissues of the fish [14].

The decline in nucleic acids (DNA and RNA) content in liver, muscle, gill tissues of freshwater fish Labeo rohita treated with malathion, due to decrease in protein synthesis and also due to liver damage, which is the major tissue for detoxification mechanism [15]. The decline in DNA content could be due to disturbances in normal DNA synthesis. The level of DNA and RNA was found to be decreased in brain, liver, gonads, and kidney of N. notopterus fish due to exposure to copper sulphate indicates toxicity effect on nucleic acid synthesis [16]. The DNA and RNA contents were increased in gill, liver, brain, and kidney of fish Channa punctatus exposed to different concentrations of cypermethrin and λ - cyhalothrin [17].

A significant decline in the DNA and RNA ratio inLabeorohita due to ammonia toxicity, and also the DNA and RNA contents were studied in the gill liver and brain of a common carp, Cyprinuscarpio exposed to cadmium chloride and lead acetate [18]. The decreased DNA content in all the tissues along with RNA content in liver and brain, but it was increased in gill due to cadmium and lead toxicity [19]. The development and growth of the fishes depend upon the DNA and RNA, which serve as biochemical indices [20]. Cellular enlargement and active protein synthesis are dependent on DNA and RNA content. Pesticides induce deoxyribonucleic acid damage [21, 22,] and structural chromosomal changes [23]. Pesticides might attack DNA directly or modify other cellular processes associated with the integrity of the genome. On exposure of cypermethrin on fish Cyprinuscarpio the DNA content decreased significantly in the brain, gill, and liver.

The reduction in DNA content is comparatively less in the liver to compared with the brain and gills. The decrement was comparatively more in gills (40%) and followed by the brain (38%) and liver (32%) [24]. Similar results were also reported in the liver and muscle tissues of Channa punctatus exposed to sublethal doses of methanol [25]. The effect of mercury and methyl parathion on the ovarian tissues of Labeorohita and observed a significant decline in the RNA: DNA ratio [26].

A decline in DNA and RNA content of the liver, brain, and muscle tissues of freshwater catfish, Clariasbatrachus exposed to endosulfan and pyrethroids [27]. DNA and RNA were declined when the animals were subjected to starvation stress [28]. Decreased DNA content was observed in whole chick embryos due to a high dose of cypermethrin, suggesting DNA damage inhibiting cell multiplication and decreasing energy supply [29]. A decline in the nucleic acid content in liver and muscle tissues of Labeorohita exposed to cypermethrin and 96 h lethal concentration(LC_{50}) of toxicant in brain, liver and kidney tissues of Labeorohita, RNA levels was decreased while DNA levels were elevated [30].

A dose-dependent decrement was observed in the nucleic acid content of the liver, muscle, and gonad tissues of freshwater teleost fish, Channa punctatus exposed to dimethoate [31]. Ribonu cleotider eductase, an enzyme erequired for the deoxyribonucleotide synthesis, might be inactivated by the free radicals generated due to exposure to the toxic compound, which might have led to the decreased levels of deoxyribonucleotide, leading to a decline in the DNA content. Oxidative damage to the nuclear material by reactive oxygen species could also have led to the decreased nucleic acid synthesis. The direct mechanism of damage involves reaction of hydroxyl radicals with DNA as a result of oxidative stress making DNA a suitable target for oxidative damage. Indirect mechanism of DNA damage involves Reactive Oxygen Species (ROS) mediated triggering of a series of metabolic events within the cell that might lead to inhibition or activation of a number of enzymes. Inhibition of enzymes that replicate or repair DNA might predispose DNA to damage. It is also possible that the disruption of DNA synthesis might affected RNA synthesis, as already suggested [32].

Pesticide compounds exhibit strong mutagenic, genotoxic, and elastogenic potentiality which is responsible for the alteration of DNA levels. However, decreased DNA is not very prominent to compare with RNA [33]. The decrease might be attributed to the increased activity of DNAase, a number of chemicals associate with DNA caused damage on liver of aquatic animals. Chemicals that cause the production of reactive oxygen species that can damage DNA, chemicals that inhibit DNA synthesis and repair. In addition, many chemical contaminants damage DNA by multiple mechanisms [34]. 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 [35]. Pesticide appears as a potential inhibitor of DNA synthesis, which might result in a reduction of RNA level [31,36].

Because of electrophilic nature, it is possible that the enzyme necessary for DNA synthesis might inhibited by toxicants. On compilation of the results, it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis. The effect of monocrotophos on *Cyprinus carpio* leads to several changes in the biochemical markers like DNA and RNA to the increased activity of the enzyme DNAase and inhibition of RNA polymerase function [37]. DNA and RNA levels increased progressively indicates a probable disruption of internal organs. The role of nucleic acids, particularly RNA/DNA and protein/DNA ratios, which are used as an index of protein synthesis and cell size, are considered as an important and form treatment with the pesticides causes variability in the nucleic acid content in different tissues, and the degree of variability or extent of alterations caused by the pesticides is found to dose-dependent. On exposure to sublethal and lethal concentrations of profenofos and carbosulfan (24 h and 8 days) to fish Labeorohita, it was found that the gill, liver, kidney, and muscle tissues the RNA content was decreased, but the brain RNA content was found to increase [38].

Present study heterogeneous levels of DNA and RNA were found in the tissues of gill, liver, kidney, brain, and muscle and the levels of DNA in different tissues indicate cell number and are constant for a species. The alterations in DNA

levels in the present study could be due to the disturbances in the normal synthesis and turnover rate of DNA besides degenerative changes. The RNA levels reflect the intensity of protein synthesis and metabolic activity of the tissue. The depletion of RNA level indicates increased proteolysis and possible utilization of the products of their degradation for metabolic purposes. Changes in the biochemical markers like DNA and RNA might be due to the increased activity of the enzyme deoxyribonuclease (DNAase) enzyme and the inhibition of RNA polymerase enzyme function. Cypermethrin reduced the DNA and RNA content in the gill, liver, and kidney of fish *Cirrhinus mrigala* [39]. Sublethal concentrations of cypermethrin showed a decrease in the level of DNA, RNA, and DNA/RNA ratio in Channa striata [40].

A decrease in DNA and RNA content is directly related to the days of exposure [41], 42. The influence of cypermethrin on *Cirrhinus mrigala* was found to time and exposure dependent for both the nucleic acids in the aquaculture practices used edible fish [43]. A significant decline in DNA and RNA contents in the gill, liver, and kidney tissues was observed in the fish *Gambusia affinis* exposed to cyfluthrin and fenvalerate [44]. DNA content was reduced to 45%, 41%, and 41% in gill, liver, and ovary of zebrafish Danio rerio, after exposure to LC40 of λ-cyhalothrin for 21 days and 51%, 53% and 55% in gill, liver, and ovary on exposure to LC_{40} of neemgold [45].

Biochemical alteration in freshwater teleost fish Colisafasciatus on exposure to synthetic pyrethroid cypermethrin for different water temperatures concentrations for 24, 48, 72 and 96 h to 0.009, 0.008 and 0.007 and 0.006 mg/L for 160 ℃ and 24, 48, 72 and 96 h to 0.06, 0.04, 0.03 and 0.02 mg/L at 280 ℃ were studied for DNA. On 96 h, exposure to 40 and 60% of 24 h LC_{50} of cypermethrin resulted in a decrease in DNA level in gonadial tissue to the value of 54 and 31% at 160 ℃ 12. In the present study, decreased levels of RNA were observed in all the tissues brain of fish exposed to sublethal concentrations of λ-cyhalothrin, whereas RNA content was increased in the brain, which might be attributed to the increased synthesis of RNA and damage in neuron cells resulting in demyelination [46].

RNA plays an important role in protein synthesis, and the inhibition of RNA synthesis at the transcription level might affect the protein content. The RNA content decreased significantly in gills (20%), brain (15%) and liver (16%) for 21 days exposure of cypermethrin on fish Cyprinuscarpio. The reduction in the RNA content was comparatively less in the brain, liver, gills and more decrement was observed in gills [24]. The decreased RNA might be due to interference in the incorporation of precursors in the nucleic acid synthesis or inhibiting the function of RNA polymerase [47]. The present observations are supported by the pesticide-mediated reduction in protein contents of various tissues, including the blood of other species of fish. DNA-damaging agents capable of inducing strand breakage, cross-links, and alkali-labile sites [48, 49, 50]. In the present study result reveals that λ-cyhalothrin caused variability in the nucleic acids content in different tissues and the degree of variability or extent of alterations found to dose dependent. Thus, similar results were also reported by various investigations: Cyprinus carpio, 24; *Cirrhinu smrigala*, 51; Channa punctatus, 52; Labeorohita, 53; Colisafaciatus, 12; *Cirrhinu smrigala*, 39; Channastriatus, 40; Channa punctatus, 17.

CONCLUSION: The present study revealed that the impact of the pesticide λ-cyhalothrin is concluded that the significant reduction in DNA and RNA content in different organs of exposed fish Ctenopharyngodonidella might be due to genotoxicity action by decreased mitotic index or due to inhibitory action of pesticides on DNA and RNA synthesis. It might also due to a decrease in protein synthesis, defective nucleic acid metabolism, and also degradation of cells.

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