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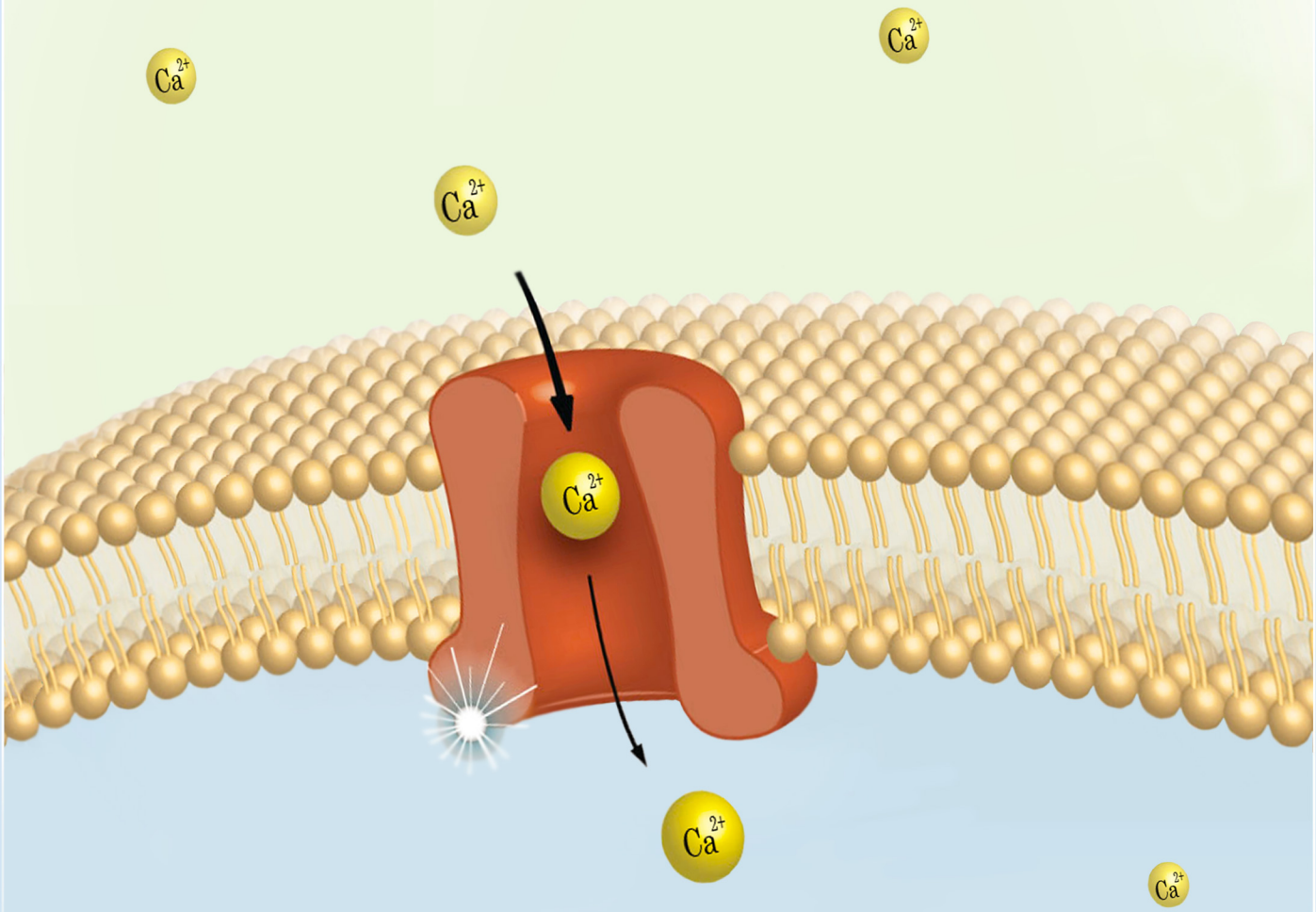

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AIM AND SCOPES

Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch- Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels, role of TRPM2 channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy, traumatic brain injury, spinal cord injury, Alzheimer' s Disease, Parkinson's Disease.

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Effects of The Hydroxyurea Derivative 1, 3 ,4 - Thiadiazoles on Antioxidant Vitamins and MDA in Serums of Rats and Cell Viability of MCF-7 Breast Cancer Cells

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ABSTRACT

In this study, the effects of as used ligand [1-hydroxy - 2 - (5 - (trifluoromethyl) - 1, 3, 4- thiadiazole- 2 - yl) guanidine] and its Mn, Cd, Cr complexes on the antioxidant vitamins and MDA levels in the serums of rats and antitumor activity of these chemical compounds on the MCF-7 breast cancer cells at different concentrations were investigated. When the results were compared between the levels of control and experimental groups of antioxidant vitamins and MDA statistically, it was observed that there were decreased levels of A, E, and C vitamins in groups of applied Cd (II) and Cr (II) complexes compared with the control group, whereas MDA levels were increased. The antioxidant vitamins and MDA levels in serum were determined by HPLC. It was clearly observed that the subcutaneously MCF-7 breast cancer cells treated Ligand (L), Mn (L) 2, Cd (L) 2 and Cr (L) 2 complexes had the low levels of cell viability activity when compared to the untreated control cells clearly increased according to the control group for after incubation 24 h onwards to 48 h. Cell vialities measured by electronic microscope. As a result, it suggested that thiadiazole compounds exhibit antitumor activity with reduction in serum antioxidant vitamins of rats can cause cytotoxic effect depending on the mechanism of oxidative damage.

Keywords: Hydroxyurea, 1, 3, 4 - thiadiazole, antioxidant, antitumor.

Introduction

1, 3, 4 - thiadiazole derivatives exhibit for example, antifungal (Kikelj D and Urleb 2002), antibacterial (Diurno et al. 1993), antimicrobial (Amorim et al. 1992), antitumor (Amir et al. 1997) and anticonvulsant properties (Jackson et al. 2007). They are very interesting compounds due to their important applications in many pharmaceutical biological and chemical fields (De Lima et al. 1994). It has also been reported that they shows antioxidative activity inhibiting lipid peroxidation (Devasagayam et al. 1983) and protein oxidation (Yoshikawa et al. 2000). Moreover, this compounds have been researched local anesthesical activities (Mazzone et al. 1993), muscle relaxant effects, nervous system depressant (Flaherty et al. 1996, Gravier et al. 1992), cytotoxicity and *in vitro* antituberkulosis activities (Barros Costa et al. 1995, Foroumadi et al. 2001).

It is said that *In vivo* experiments showed that its antitumor and immunosuppressive actions were about 5 times higher than those of the parent compound, 2-amino-l,3,4-thiadiazole (NSC 4728), on a weight basis. That is, NSC 143019 was effective against L1210 leukemia, 6C3HED/OG lymph sarcoma, C1498 myeloid leukemia, Ehrlich carcinoma, Sarcoma 180, B16 melanoma and X5563 myeloma at nontoxic doses in BALB/3T3 mice (Matsumoto et al. 1974).

It is said that effective antitumor candidate drugs in the aspect of MCF-7 cytotoxicity and Ni-NQTS were statistically the most effective (Chen et al. 2004). It has been determined that some class of thiadiazoles applied on rats peroxisome proliferator activated in lipid metabolism (Steppan et al. 2002), decreased level of free fatty acid (Miyazaki et al. 2001) and increased level of insulin in liver (Mazzone et al. 1993).

In a study, carried out by Karagozoglu et al. (2013) investigated the antioxidant and antihepatotoxic effect of hydoxyurea derivative 1,3,4-thiadiazoles on serum biochemical parameters (AST, ALT, LDH, urea, creatinine and total bilirubin) and antioxidant parameters (SOD, CAT, GP_X, MDA). In this study, the levels of AST and GP_X were found to be lower in the Cr-L group than in other groups. Moreover, while the levels of ALT and total bilirubine were found to be lower, the level of SOD was found to be higher in the Cd-L group than in other groups. In another study, carried out by Turkoglu et al. (2014) anaylzed effects of thiadiazole ligand and its metal complexes (Mn, Cr and Cd) on the fatty acids and lyphophlilic vitamins of in the liver of rats and it was observed that the amount of α-tocopherol increased both in the Mn and Cr complexes groups when compared to the control group but this increase was parallel to the amount in the same groups. On the other hand the amount of retinol was found to be lower in the Mn complex group than in other groups.

In the present study, the effect of hydroxyurea derivative 1, 3, 4-thiadiazoles and its metal complexes on the levels of antioxidant vitamins (A, E, C) and MDA, as indicator of lipid peroxidation in serum of rats which is injected subcutaneously and antitumor activity in cell culture media were investigated. Analysis of antioxidant vitamins and MDA were determined by high pressure liquid chromatography. The *in vitro* antitumor activity of these compounds on MCF-7 breast cancer cells in culture medium were investigated. Cell vitalities measured by electronic microscope.

Materials and Methods

Animal Treatments

In the study 35 adult male wistar rats, raised in Fırat University Faculty of Medicine Experimental Research Center and in an average weight of 250 g, were used as animal material. Rats were kept for 12 hours in the light and for 12 hours in the dark at room temperature. Water and feed were given to rats as required. Experimental protocol was approved by the Ethics Committee of Fırat University Animal Experiments. The study was carried out in accordance with the rules. Thiadiazole complexes were diluted with corn oil in a way that its amount would be below % 10 as dimethylsulfoxide (DMSO) also dissolved (Varvaresou et al. 2000). Animals were divided into 1 control group and 4 implementation groups including 7 for each. DMSO, diluted with only corn oil, was injected to the control group. 0.5 ml DMSO including 25 mg / kg was injected subcutaneously to ligand and the other metal complex groups for 15 days with three-day interval during the test (Cesur et al .2002).

Chemical Compounds

Hydroxyurea derivative 1,3,4- thiadiazole compounds and their metal complexes used in the applications were synthesized and characterized by Çetin et al. (Çetin et al. 2006). The structure of ligands and their complexes are below (Fig. 1).

Analytical Methods

Determination of Serum Vitamin C and MDA Levels

A volume of 0.3mL of serum sample was taken then 0.3 ml of 0.5 M $HClO_4$ was added for precipitated proteins. This mixture was then vortexed and pure water was added to the total 1 ml volume. After 15 minutes, the mixture centrifuged (2500 rpm / min) and then 20 µl samples were carefully taken from above supernatants and injected on the HPLC. The detection was performed at 254 nm for vitamin C and MDA. Finally results were calculated as µg / ml for MDA and C vitamin (Karatepe et al. 2004).

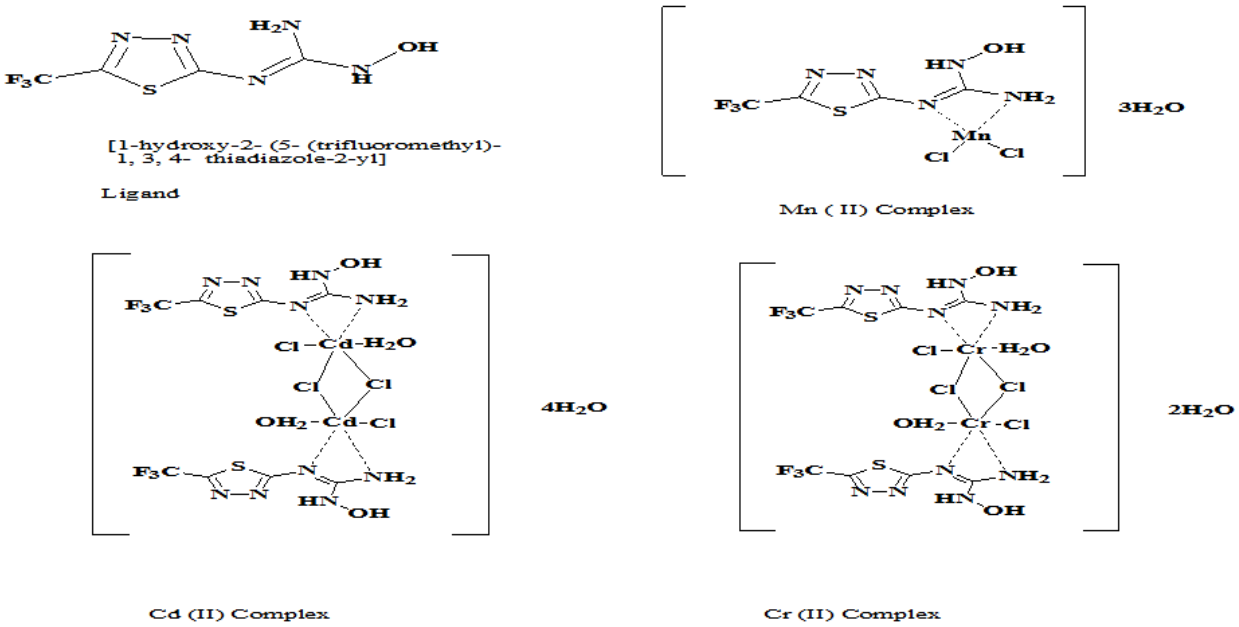

Figure1. Chemical structure of ligand and its complexes

Determination of Serum Vitamin A and E Levels

0.3 ml of serum samples was taken and another 0.3 ml of ethyl alcohol (containing 1% H_2SO_4) was added for precipitated proteins. After vortexing, the samples centrifuged at 2500 rpm of 5 min. following the centrifugation fase, 250µl n-hexane added and the tubes were vortexed and centrifuged once again. At the end of centrifugation, the hexane mixture was taken carefully to a glass tube and 250 µL of hexane were added and centrifuged one more time. Hexane formula caused to evaporate with nitrogen flow, and the residue was made to dissolve in a 100 µL of methanol and 20 µL of sample were injected into the HPLC system. The detection was performed at 326 for vitamin A and 296 nm for vitamin E. In the end, the results were calculated as µg / mL vitamin A and vitamin E (Catignani and Bieri 1983; Henning et al. 1997).

Antitumor Activity

Cell line and growth conditions

The human MCF -7 breast cancer cell line used in this study was obtained from the American Type Cell Culture Collection. The cells were grown in Dulbecco's (Seromed, Germany) minimal essential medium (DMEM) enriched with 10% foetal calf serum (FCS; Biochrom, Germany), 100 mg mL^{-1}streptomycin and 100 IU mL^{-1}penicillin in a humidified atmosphere of 5% CO_2 at 37°C. The cells were harvested using trypsin (0.05%:0.02%) solution.

Assessment of cell proliferation

For the cytotoxicity analysis with the test compounds, cells were seeded at 1×10^6 mL^{-1} of MCF-7 cells per eppendorf tubes in six-replicates. The test compounds were solved in DMSO. Proliferative MCF-7 cell lines were seeded in flasks and incubated for 24 h. After pre-incubation, the cell culture medium was replaced fresh medium and each test compound was added to the medium within the range of 7.5 and 100 µM, and the cells were incubated in an air humidified incubator at 37°C in 5% CO_2 24 and 48 h. After incubations, cell viability was measured using trypan blue exclusion method (Fener et al. 1993; George et al. 1996).

Statistical Analysis

All data were expressed as means ± SD. Standard statistical analyses were done, including one-way anova with tukey test for multiple comparisons of groups and taking $p< 0.05$ as the level of significance.

Results

Antioxidant Vitamins (A, E, C) and MDA

The results of the levels of antioxidant vitamins (A, E, C) and MDA in the serum of chemical substances treated rats are given as a showing comparison of

application groups with the control group in each of the parameter in the table 1 below. The levels of vitamins A, E, C and MDA in serum of the Thiadiazole compounds (TDAC) are presented in Table 1. The percentages of $Mn(L)_2$, Ligand(L), $Cr(L)_2$, $Cd(L)_2$ levels decreased 41.55%, 41.99%, 47.18%, 76.19% respectively for vitamin A ($p<0.05$) and 23.53%, 23.89%, 44.05%, 52.56% respectively for vitamin E ($p<0.05$) in comparison with the control group. On the other hand the percentages of $Mn(L)_2$, Ligand(L), $Cd(L)_2$, $Cr(L)_2$ levels increased by 6.44%, 3.95% 13.51% and 14.13% ($p<0.01$) respectively for MDA in comparison with the control group.

In table 1, it was clearly observed that the rats subcutaneously injected TDAC had the low levels of antioxidant vitamins A, E and C in rat serum when compared with the control group ($p < 0.05$). On the other hand, MDA levels clearly increased according to the control group ($p < 0.01$).

Antitumor Activity

The antitumor level of dose (7,5μM, 15μM, 30μM) and time (24 h, 48 h) dependant of the Thiadiazole compounds (TDAC) are presented in table 2. Treatment of Ligand(L), $Mn(L)_2$, $Cd(L)_2$, $Cr(L)_2$ with 7,5μM concentration reduced the cell viability of MCF-7 cells (26.38 - 64.16%, 29.53 - 59.88%, 28.31 - 66.39%, 29.53 - 63.13%, $p <0.05$) respectively from after incubation 24 h onwards to 48h. Treatment of Ligand(L), $Mn(L)_2$, $Cd(L)_2$, $Cr(L)_2$ with 15μM concentration reduced the cell viability of MCF-7 cells (57.23 - 74.04%, 40.12 - 70.07 %, 43.79 - 78.20%, 53.87 - 74.95%, $p <0.05$) respectively from after incubation 24 h onwards to 48h. Treatment of Ligand(L),, $Mn(L)_2$, $Cd(L)_2$, $Cr(L)_2$ with 30μM concentration reduced the cell viability of MCF-7 cells (61.53 - 78.62%, 66.09 - 81.46 %, 67.32 - 88.62%, 59.37 - 83.08%, $p <0.05$) respectively from after incubation 24 h onwards to 48h. In table 2, it were clearly observed that the subcutaneously MCF-7 breast cancer cells treated TDAC had the low levels of cell viability when compared to the untreated control cells ($p < 0.05$).

Discussion and Conclusion

Reactive oxygen species, particularly free radically induced lipid peroxidative tissue damage, have been implicated in the pathogenesis of various diseases. Lipid peroxidation is assessed in an indirect manner by the measurement of secondary products, such as malondialdehyde (MDA) (Henning et al. 1997). Vitamin A has multiple functions: Firstly, it is important for growth and development, as well as the maintenance of the immune system and good vision (Albers et al. 1999). Both vitamin E and C react rapidly with organic free radicals, and it is widely accepted that the antioxidant properties of these compounds are partially responsible for their biological activity. Nevertheless, vitamin E is considered more lipophilic than vitamin C, and it has been found to be the most potent antioxidant in bio-membranes, particularly with respect to lipid peroxidation. Penetration into a precise site in the membrane is probably a significant characteristic of the protection against highly reactive radicals (Slater 1978; Packer et al. 1979).

In this study, it was not observed a significant change in levels of vitamin C. Vitamins E level in the ligand and $Mn(L)_2$ groups was similar to the control group. While vitamin A and E levels in the $CdL)_2$ and $Cr(L)_2$ groups were lower, the MDA level in the their groups was higher than among the other groups. However, the results of obtained from $Cd(L)_2$ were similar to results observed with the Cr(II) complex. Serum MDA level is increased and vitamins A and E levels are decreased by $CdL)_2$ and $Cr(L)_2$ treatments. Detailed studies in the past two decades have shown that metals like cadmium (Cd), chromium (Cr) possess the ability to produce reactive radicals, resulting in DNA damage, lipid peroxidation, depletetion of protein sulfhydryls and other effects. Reactive radical species include a wide range of oxygen-, carbon-, sulfur-radicals, originating from the superoxide radical, hydrogen peroxide, and lipid peroxides but also in chelates of amino-acids, peptides, and proteins complexed with the toxic metals. The toxic effects of metals involve hepatoxicity, neurotoxicity and nephrotoxicity (Valko et al. 2005).

Table 1. Levels of antioxidant vitamins (A, E and C) and MDA in serum in Thiadizaoles Compounds (TDAC) and control groups in rats

TDAC (n=7)	Vitamin E (mg/lt)	Vitamin A (mg/lt)	Vitamin C (mg/lt)	MDA (mg/lt)
Control	2.808±0.139	0.231±0.018	21.977±1.891	0.481±0.010
Ligand	2.277±0.138 *	0.134±0.003*	18.922±0.347*	0.510±0.011**
$Mn(L)_2$	2.267±0.126 *	0.135±0.008*	17.471±0.275*	0.500±0.012**
$Cd(L)_2$	1.472±0.140*	0.055±0.004*	17.161±0.227*	0.546±0.026**
$Cr(L)_2$	1.631±0.107*	0.122±0.004*	17.308±0.237*	0.549±0.024**

* : $p<0.05$,** : $p<0.01$, Ligand: L

Table 2. For 24 (a) and 48 (b) hours effect of 7,5 µM, 15 µM, 30 µM Thiadizaoles Compounds (TDAC) on MCF-7 breast cancer cells

(a) Cell viability of MCF-7 cells after treatment at 24 hours

TDAC (n=7)	7,5µM	15µM	30µM
Control	81.83±1.833	81.83±1.833	81.83±1.833
Ligand	60.33±1.606*	35.00±2.516 *	31.50±3.013*
Mn(L)2	57.67±2.011*	49.00±1.471*	27.75±3.682*
Cd(L)2	58.67±2.044*	46.00± 4.708*	26.75±3.092*
Cr(L)2	57.67±2.201*	37.75±1.796*	33.25±2.136*

(b) Cell viability of MCF-7 cells after treatment at 48 hours

TDAC (n=7)	7,5µM	15µM	30µM
Control	76.83±2.120	76.83±2.120	76.83±2.120
Ligand	29.33±2.565*	21.25±7.192*	17.50±3.068*
Mn(L)2	30.83±1.352*	23.00±1.957*	14.25±0.816*
Cd(L)2	25.83±1.222*	16.75±4.767*	8.75±3.567*
Cr(L)2	28.33±4.752*	19.25±2.250*	13.00±0.577*

* : $p<0.05$, Ligand: L

The results of the our antioxidant study are likely to be seen in findings of one study reported that 4-(1-phenylmethylcyclobutane-3-yl)-2-(2-hydroxybenzyli-denehydrazino) thiazole (LH) at a dose of 150 mg kg^{-1} were no change in the levels of their serum vitamin A, E, C and MDA, but the Cd (II) complex decreased serum vitamin A and E levels by increasing the serum MDA level in rats (Oner et al. 2005). Similarly, it has been reported that 4- (1-phenylmethylcyclobutane-3-yl)-2-(2-hydroxybenzylidenehydrazino) thiazole at a dose of 25 mg kg^{-1} did not change serum vitamin A, E, C or MDA levels, and its Zn(II) complex increased only serum vitamin E levels. The Cu(II) complex decreased serum vitamin A and E levels and increased the serum MDA level while all compounds affected some free amino acids by increasing isoleucine, tryptophan and methionine levels in rats (Karatepe 2002).

The results of present study showed that the rate of growth of MCF-7 cells strongly affects the antiproliferative action of physiological doses of hydroxyurea derivative 1, 3, 4 - thiadiazoles on these human tumoral cells. It was shown that levels of serum antioxidant vitamin A and E had the very low in Cd complex group. Moreover, it was observed that group of Cd (II) and Cr (II) complexes exhibited higher antitumor activity against to MCF -7 human breast cancer cells than other groups. These results suggested that thiadiazole compounds exhibit antitumor activity with reduction in serum antioxidant vitamin of rats can cause cytotoxic effect depending on the mechanism of oxidative damage. It can be think that ligand and its metal complexes exhibite antioxidant / pro-oxidant effects in vivo and they may lead to cytotoxic activity on the MCF -7 human breast cancer cells in vitro culture system.

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