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THE EFFECTS OF HYDROXYUREA DERIVATIVE 1, 3, 4- THIADIAZOLES ON SERUM BIOCHEMICAL PARAMETERS AND ANTIOXIDANT STATUS IN LIVER OF RATS

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In this study, it was aimed to investigate 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, GPX, MDA). In our study, a total of 35 adult male wistar rats were divided into 5 equal groups. DMSO diluted with only corn oil, was injected to the control group. 25 mg/kg ligand, 25 mg/kg thiadiazole-manganese, 25 mg/kg thiadiazole-cadmium and 25 mg/kg thiadiazole-chrome complexes was injected to the experimental groups rats subcutaneously for 15 days with three-day interval during the test. After blood samples were centrifuged AST, ALT, LDH enzyme activites hepatic injury; and then, the activites of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and the level of malondialdehyde (MDA) in liver tissues were determined spectrophotometrically. The level of AST was found to be lower ($p<0.05$) in the Cr-L group than in other groups. The levels of ALT and total bilirubine were found to be lower ($p<0.05$) in the Cd-L group than in other groups. The level of SOD was found to be higher ($p<0.05$) in the Cd-L group than in other groups. The levels of GPX was found to be lower ($p<0.05$) in the Cr-L group than in other groups. As a result, it can be concluded that their pharmacological characteristics can be beneficial in many fields of application because hydroxyurea derivative 1,3,4 thiadiazole compounds show the antioxidant and antihepatotoxic activity.

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Keywords: Hydroxyurea, Thiadiazoles, Serum, Biochemical Parametres, Antioxidant Status, Liver

1. Introduction

Thiadiazole derivatives are used therapeutically in various diseases due to the properties such as antifungal [1], antiviral [2], antibacterial [3], anticonvulsant [4], antimicrobial and anti-inflammatory [5]. It is also stated that thiadiazole derivatives show antityroid activity [6]. It is determined in the studies conducted as *in vivo* that some thiadiazole derivatives (2, 2 - bis-1, 3, 4 – thiadiazole) show antitumor and immunosuppressive activity against types of leukemia such as L1210 leukemia 6C3HED/OG lymphosarcoma, C1498 myeloid leukemia, Ehrlich carcinoma, sarcoma 180, B16 melanoma and X5563 myeloma in BALB/3T3 rats [7]. Especially, it was

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investigated that N-substituted 2-amino-1, 3, 4-thiadiazole derivatives showed antiproliferative activity [8]. It was observed *in vivo* studies [9] that thiadiazole ligands inhibited the enzyme carbonic anhydrase (CA, EC 4.2.1.1). Also, it is stated that they show antioxidative activity by inhibiting lipid peroxidation [10], protein oxidation [11].

It was reported that Cd (II) metal complex acted as antioxidant by creating an oxidative stress and caused damage on the testicular tissue when the Schiff base derivative Cd (II) metal complex comprising thiosemicarbazone derivative was injected into rats in high doses [12].

In a study that Karatepe and his friends conducted, it was stated that ligand synthesized from thiazole compounds of the Schiff base derivative did not affect the antioxidant parameters, Cu (II) complex, and Cd (II) complex acted as an oxidant. They stated that Zn(II) complex did not create any oxidative stress, however it acted as an oxidant, did not cause any damage on adren tissues [13].

It is known chemicals taken into the body create changes in the routine serum biochemical blood parameters. Aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH), total bilirubin, urea and creatine are among the biochemical indicators of the organs' functions. And it is used as biomarkers of oxidative stress free radical scavenging enzyme activities like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and thiobarbituric acid reactive substances (TBARS).

In this study, it has been aimed to investigate the effects of hydroxyurea-derived 1, 3, 4 - thiadiazole compounds, known to have many biological activities, on serum biochemical parameters (AST, ALT, LDH, total bilirubin, urea nitrogen and creatinine) and antioxidant enzyme activities (SOD, CAT and GPX) and lipid peroxidation in liver of rats.

2. Material and method

Experimental Protocol: 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 [14]. 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 [15].

Chemicals: Hydroxyurea derivative 1,3,4- thiadiazole compounds and their metal complexes used in the applications were synthesized and characterized by Çetin et al. [16]. The structure of ligands and their complexes are below (Fig. 1).

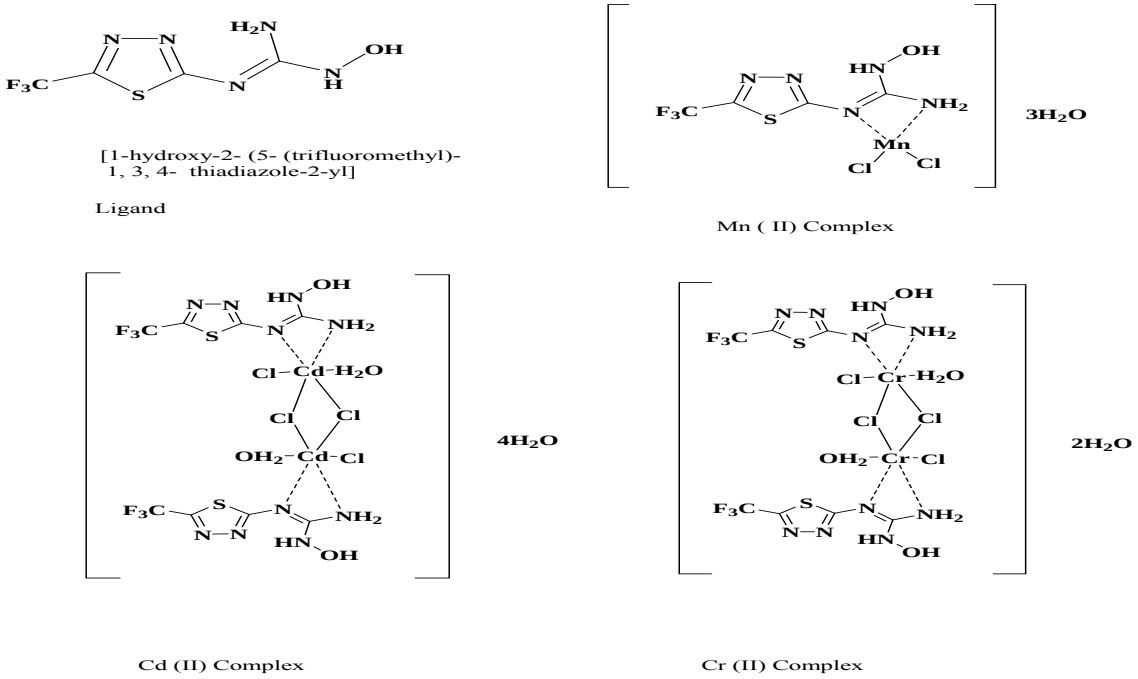

Fig. 1. Chemical structure of ligand and ıts complexes

Serum biochemical parameters: Collected blood was used for the estimation of serum biochemical parameters. Homogenate was centrifuged at 3.500 rpm for 10 min at 4 ^{o}C. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) enzyme activities and urea, creatinine and total bilirubine levels were measured by using commercially available kits (Span Diagnostic Ltd., Surat, India). The analyses were carried out according to the manufacturer's instructions. Data were obtained using an Dimension RxL max auto-analyzer (Siemens Healthcare Diagnostics Ltd.)

Liver biochemical parameters

Estimation of Superoxide Dismutase (SOD): Method of Muradian et al [17] used for estimation of SOD which detects O_2^- by oxidation of hydroxylamine HCl to 2 nitrite. The coloured product was measured calorimetrically at 560 nm. 100 µl of 5 % liver homogenate in 0.2 M sucrose was added into 1 ml of sodium carbonate, 0.4 ml of nitro blue tetra zolam (NBT) and 0.2 ml of EDTA and zero minute reading was taken at 560 nm. 0.4 ml of hydroxylamine (1 mM) was added in the reaction mixture and after incubation for 5 min. at 25°C, the resulting coloured product was measured colourimetrically at 560 nm. SOD activity was estimated in units/mg protein as the quantity of protein in 1000 µl of 5 % liver homogenate that inhibited reduction of 24 mM NBT by 50 %.

Estimation of Catalase (CAT): Method of Sinha [18] was followed to estimate decomposition of hydrogen peroxide by tissue catalase. 100µl of 5 % liver homogenate in 0.15 M KCl was added in 1.9 ml of phosphate buffer (0.25 M, pH 7) and absorbance was measured at 240 nm. 1 ml of hydrogen peroxide solution (0.34 ml of 30 % H_2O_2 in 100 ml distilled water) was added in the 2 2 reaction mixture and absorbance was measured after 1 minute at 240 nm. The catalase activity was expressed as U/mg of protein. 1 international unit of CAT is the amount that catalyses 1 mM of H O per minute at 37°C.

Estimation of Glutathione Peroxidase (GPX): Method of Alexander [19] was followed for estimation of glutathione peroxidase (GPX) from tissue homogenate. Extent of periodide formation proportional to the GPX concentration in reaction mixture was determined from absorbance measurements at 353 nm. 0.5 ml of tissue homogenate in 0.1 M KCl was mixed with 1 ml KI solution and 1 ml of sodium acetate (0.1M, pH5.25) and absorbance was measured colourimetrically after 5 min. of incubation at 353 nm. 200µl of H_2O_2 was added in the reaction mixture and absorbance is 2 2 measured after 5 minutes of incubation at room temperature at 353

nm. 1 unit of GPX activity corresponds to change in 1 OD per minute and expressed as U/mg protein.

Estimation of Lipid Peroxidation: Lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) was estimated as per the method of Fraga et al [20] 1.0 ml of the sample extract was added with 2.0 ml of the TCA- TBA- HCl reagent (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl). The contents were boiled for 15 minutes, cooled and centrifuged at 10,000 rpm to remove the precipitate. The absorbance was read at 535 nm and the TBARS value was expressed as nmol of malondialdehyde (MDA) equivalent per gram of tissue.

Statistical Analysis: All results were found by means of SPSS 15.0 (SPSS Inc., Chicago, IL, USA) statistical program by using the mean ± standard derivation (SD). The results were analyzed for statistical significance by one-way ANOVA followed by Duncan's post hoc test of significance. $P < 0.05$ was considered as statistically significant.

3. Results

Serum biochemical parameters (urea, creatinine, total bilirubin, AST, ALT, and LDH) of the rats to which thiadiazole complexes were applied and the control group rats have been presented in the Table I. The level of AST, ALT and total bilirubine slightly decreased ($p<0.05$) in all groups when compared to control. However, there was no significant difference the levels of LDH, creatinine and urea in all groups when compared to the control group ($p<0.05$). The level of AST was found to be lower ($p<0.05$) in the Cr-L group than in other groups. The levels of ALT and total bilirubine were found to be lower ($p<0.05$) in the Cd-L group than in other groups.

Liver biochemical parameters (SOD, MDA, CAT, GPX of the rats to which thiadiazole complexes were applied and the control group rats have been presented in the Table II. The level of SOD slightly increased ($p<0.05$) in all groups when compared to control. The level of GPX slightly decreased ($p<0.05$) in all groups when compared to control. However, there was no significant difference the levels of MDA and CAT in all groups when compared to the control group ($p<0.05$). The level of SOD was found to be higher ($p<0.05$) in the Cd-L group than in other groups. The levels of GPX was found to be lower ($p<0.05$) in the Cr-L group than in other groups.

Table-I. Effect of The Hydroxyurea Derivative 1, 3, 4 – Thiadiazoles on Serum Biochemical Parameters in Rats

Parameters (n =7)	**Groups**					***P***
	Control	***Ligand***	***Mn-L***	***Cd-L***	***Cr-L***	
AST	250.25±73.82	156,25±4,50 [b]	169,25±27,49 [abc]	184,25±17,39 [a]	145,5±12,56 [bc]	*P<0,05*
ALT	28,75±15,39	12,75±3,20 [c]	19,00±1,15 [ab]	7,75±1,71 [d]	18±1,83 [a]	*P<0,05*
LDH	1998,5±334,15	2135,5±208,6	1875,75±392,4	1859,50±87,5	1573,8±291,8	*P>0,05*
Creatinine	0,83±0,33	0,65±0,29	0,73±0,21	0,48±0,05	0,8±0,36	*P>0,05*
Urea	41,75±13,77	25,75±4,65	34,50±5,80	25,75±2,87	27,75±8,96	*P>0,05*
Total Bilirubine	0,21±0,02	0,17±0,04 [a]	0,18±0,01 [ab]	0,12±0,02 [acd]	0,15±0,03 [ac]	*P<0,05*

Mean ± Standard Deviation(SD)
Each mean represents analyses of five independent samples (a, b, c, d) Variation in the following letters between samples indicates significant of difference by Duncan's test at 5% level ($p<0.05$), P: Statistical values, L:Ligand

Table II. Effect of The Hydroxyurea Derivative 1, 3, 4 – Thiadiazoles on Liver Biochemical Parametres in Rats

Parameters (n =7)	Groups					P
	Control	*Ligand*	*Mn-L*	*Cd-L*	*Cr-L*	
SOD	143,82±1,15	154,71±3,68[d]	182,65±1,02[b]	190,34±1,14[a]	175,79±2,24[c]	***(P<0,05)***
MDA	1,94±0,12	1,95±0,06	2,20±0,11	2,28±0,14	2,15±0,08	*P>0,05*
CAT	69,46±0,85	73,37±0,98	70,34±0,57	71,72±0,5	73,74±0,87	*P>0,05*
G-Px	17,78±0,62	14,98±0,59[b]	14,24±0,47[c]	15,01±0,22[b]	14,23±0,38[c]	***(P<0,05)***

Mean ± Standard Deviation(SD)
Each mean represents analyses of five independent samples (a, b, c, d) Variation in the following letters between samples indicates significant of difference by Duncan's test at 5% level ($p<0.05$), P: Statistical values, L:Ligand

4. Discussion

Blumberg et al reported that there had been increases in the amounts of AST, ALT activities in the rats to which compounds containing 5-substituted 2-aminothiazol ring [21]. Kara and Servi reported in a study they conducted that serum AST and ALT activities increased and the level of BUN (blood urea nitrogen) decreased in the mice to which prednisolone was given with 1.8 mg / kg / day cadmium chloride [22]. In a study in which 6-substituted 1, 2, 4-triazole-[3,4-b] - 1, 3, 4-thiadiazole 1, 3, 4 - oxadiazole compounds of isoniazid were used, it was reported that there had been a rise in the amounts of SGOT (AST) and SPGT (ALT) activities, also a decrease in the MDA level, an indicator of the level of hepatotoxic lipid peroxide, in the comparison of the control group and implementation groups [23].

Adediji et al were prepared and characterized and evaluated biological activity of 2,5-diamino-1,3,4-thiadiazole, derived from semicarbazide hydrochloride, and its metal complexes. They reported that there had been increases in the levels of ALT activities in the rats to which compounds containing Cu and Ni complexes compared to the ligand and Co complex groups. However there had been no significant difference between control group and all the other groups in AST parameter [24].

El-Naggar et. al evaluated the anti-tumour activity of some newly synthesized heterocyclic 1,3,4-thiadiazole and 1,2,4-triazine derivatives. Different groups of mice were inoculated with Ehrlichs Ascites Carcinoma cells (EAC) intra-peritoneal. After one day of inculcation, mice were treated with twenty five different new derivatives of 1,3,4-thiadiazoles or 1,2,4-triazines. Only five compounds of 1,3,4-thiadiazoles namely 3,4,5-triacetoxy-6-(5-amino--[1,3,4] thiadiazol- 2-ylsulfanyl)-tetrahydro-pyran-2-ylmethly ester (Cpd.1), N-(5-mercapto-1,3,4-thiadiazol-2yl)-2(phenylsulfonamido) acetamide (Cpd.7), 2-thiono-1,3,4-thiadiazolyl-butane-1,4-sulfam (Cpd.12), 2-(5-(5-oxo-2-phenyloxazolidin-3-yl)-1,3,4-thiadiazol-2-ylthio) acetic acid (Cpd.15) and 2-(5-(5-oxo2-(4-chlorophenyl)oxazolidin-3-yl)-1,3,4-thiadiazol-2ylthio) acetic acid (Cpd.16) significantly decreased the total ascetic volume as compared to the untreated tumour bearing mice. The anti-tumour activity of these derivatives against EAC-bearing mice were monitored through the changes in some biochemical parameters. This study showed that AST enzyme increased in both of the untreated group (tumour alone) and cisplatin – treated mice. In contrast, groups of mice which treated with Cpd.12 or Cpd.15, showed non-significant changes in AST as compared with naive mice. These results could explain that these compounds have no remarkable liver toxicity. Interestingly, ALT activity decreased in EAC-bearing and in all groups treated with new synthetic compounds as compared with naive mice. Furthermore, as compared to the naive mice, the level of

creatinine and urea increased in all the treated groups with new synthetic derivatives as well as in the untreated EAC-bearing mice [25].

CAT, SOD, GR and GPX are examples of enzymatic antioxidants. SOD and CAT considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species. SOD is an important defence enzyme, which catalyzes the dismutation of superoxide radicals and said to be the first enzyme that responds against oxygen radicals [26]. CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals. SOD and CAT are present in all oxygen-metabolizing cells and their function is to provide a defence against the potentially damaging reactivities of superoxide and hydrogen peroxide [27].

In the study of El 0Naggar et. al, levels of GSH were found to be significantly reduced in cisplatin or treated with Cpds. 1,7,15 or Cpd.16, when compared with naive mice. The level of SOD and catalase were decreased in all other groups as compared to the naive mice [25].

5. Conclusion

The decrease in GPX activity causes the increase of H_2O_2 levels and cell injury. CAT enzyme protects the cells the oxidative damage depending on H_2O_2 by breaking into pieces H2O2 that emerges by way of SOD to oxygen and water. However if in this ambient there are more superoxide, this can inhibit CAT and GPX. Thus the increase in SOD can be an indicator of more superoxide radical in the ambient. However the increase in both SOD and CAT activity can be accepted as an enzymatic defence for protecting the cell against lipid peroxidation in removing the free radical. In this study although there is the increase in liver MDA level, the increase in CAT and SOD activity can show that the growth of oxidative stress in infection period and the balance between anti oxidant and pro- oxidant disorders for the benefit of antioxidant.

That there is not any increase in AST and ALT enzyme activity indicates that this will not cause the liver damage. In other words, it can be said that these compounds make much less toxic effect, in addition they create protective effect in liver toxicity.

In conclusion it is thought that their pharmacological characteristics can be benefited in many field of application because hydroxyurea derivative 1,3,4 thiadiazole compounds show the antioxidant and antihepatotoxic activity.

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