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# Biochemical evaluation of hydroxyurea derivative schiff bases in liver of rats

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**Abstract:** In this study, it was aimed to examine the antioxidant and antihepatotoxic effects of hydroxyurea derivative Schiff bases on serum biochemical parameters (AST, ALT, LDH, urea, creatinine and total bilirubin) and antioxidant parameters (SOD, CAT, GPx, MDA). In this study, a total of 49 adult male Wistar rats was examined and they were divided into 7 equal groups. DMSO, which is diluted only with corn oil, was administered to control group. 25 mg / kg ligand, 25 mg / kg Schiff base - manganese, 25 mg / kg Schiff base-copper, 25 mg / kg Schiff base - zinc, 25 mg / kg Schiff base - nickel, 25 mg / kg Schiff base - cobalt complexes were administered to rats of experimental group subcutaneously for 15 days with three-day intervals throughout the test process. All specimens were killed by decapitation and their livers were extracted. According to the results obtained, ALT level was observed to be higher ($P<0.05$) in the Cu-L group compared to other groups. LDH level was observed to be higher ($P<0.05$) in the Cu-L and Co-L groups compared to other groups. SOD level was observed to be higher ($P<0.05$) in the Cu-L, Mn-L and Zn-L groups compared to other groups. MDA level was observed to be higher ($P<0.05$) in the Ni-L, Cu-L, Zn-L groups compared to other groups. In conclusion, it can be suggested that the determination of the pharmacological characteristics of them can be beneficial in numerous fields of application thanks to the antioxidant and hepatotoxic activities demonstrated by hydroxyurea derivative Schiff bases.

***Key words:*** Hydroxyurea; Schiff bases; Serum; Biochemical parameters; Antioxidant enzymes; Liver.

## Introduction

Schiff bases and their metal complexes are utilized therapeutically in various diseases thanks to their features such as antitumoral (1), antiviral (2), antimicrobial (3) and antineoplastic properties. Additionally, it was suggested that Schiff bases and copper (II) complexes demonstrated antioxidative activities by preventing lipid peroxidation (4). Four coordinated Co (II) Schiff base chelate complexes demonstrate catalytic activities in oxygenation of alkene (5) and synthetic iron (II) Schiff base complex demonstrate catalytic activities for electro-reduction of oxygen (6). A number of Schiff base holds anti-inflammatory, allergic inhibitors reducing activities and radical scavenging (7), analgesic (8) and anti-oxidative actions (9).

Schiff bases derived from thiazole derivatives were suggested to possess notable anticancer activities (10). Numerous anticancer drugs are viable ligands (11). Several of these drugs prevent elevated anticancer activities when they are administered as metal complexes (12, 13). Physical features and antifungal activities of various Schiff bases derived from 4-aryl-2-aminothiazoles, substituted 2-aminobenzothiazoles, 4-aryl-2-aminothiazole methiodides, 4-aryl-2-substituted-methylaminothiazoles or 2-(2- hydroxyphenyl or naphthyl)-3-(4-arythiazol-2-yl)-4-thiazolidones and vanillin were comprehensively investigated (14, 15). Sixteen Schiff base complexes of Co, Ni, Cu, and Zn derived from substituted thiazoles and substituted salicylaldehydes were produced and examined for their antineoplastic potencies against L-1210 lymphoid leukemia (16).

In a study conducted by Karatepe and Karatas, it was reported that the rats were administered subcutaneously with a new thiosemicarbazone (HL) and its $CuL_2$ and $ZnL_2$ complexes. In that study, it was aimed to determine the effects of new compounds on serum antioxidant vitamins (A, E, C), selenium (Se), malondialdehyde (MDA) levels, erythrocyte GSH-Px enzyme activities and morphological changes in the liver, kidney and adrenal gland tissues. It was reported that erythrocyte GSH-Px activities, serum MDA and vitamins A, E concentrations were alternated statistically while serum levels of selenium, and vitamin C were not altered. As a result, the measured parameters demonstrated that $CuL_2$ resulted in significant oxidative stresses and $ZnL_2$ acted as an antioxidant (16).

It is a well-known fact that chemicals which enter the body cause alterations in the routine serum biochemical blood parameters. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin, urea, and creatinine are recog-

nized as biochemical indicators of the organs' functions among others and they are utilized as biomarkers of oxidative stress free radical scavenging enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and thiobarbituric acid reactive substances (TBARS).

In this study, it was aimed to investigate the effects of hydroxyurea derivative Schiff bases, which are recognized to possess numerous 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.

## Materials and Methods

### Experimental protocol

In this study, 49 adult male Wistar rats, which were raised in the Experimental Research Center of the Faculty of Medicine at Fırat University with an average weight of 250 g, were adopted as specimen material. Rats were kept for 12 hours in the light and for 12 hours in the dark, at room temperature. Water and feed were provided ad libitum. The Experimental protocol adopted in the study was approved by the Ethics Committee ofFırat University Animal Experiments. The study was conducted according to the rules. Hydroxyurea Schiff base complexes were diluted with corn oil in a way that ensured its amount to be below 10% because, in the process, dimethylsulfoxide (DMSO) was dissolved, too (18). Specimens were divided into 7 equal groups which consisted 1 control group and 6 experimental groups which included 7 specimens each. DMSO, diluted only with corn oil, was administered to control group. 0.5 ml DMSO including 25 mg/kg was administered subcutaneously to ligand and the other metal complex groups for 15 days with three-day intervals throughout the test process (19).

### Chemicals

Hydroxyurea derivative Schiff base compounds and their metal complexes, which were employed in the practices were synthesized and characterized by Sekerci et al. (20, 21, 22). The structure of ligands and their complexes were demonstrated below (Fig. 1).

### Serum biochemical parameters

The blood collected was utilized in the determination of serum biochemical parameters. The homogenous material was subjected to centrifuging at 3.500 rpm for 10 min at 4 °C. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) enzyme activities along with urea, creatinine and total bilirubin levels were determined by practicing with commercially available kits (Span Diagnostic Ltd., Surat, India). The instructions of the manufacturer were followed in the conducted analyses. The obtained data were gathered with a Dimension RxL max auto-analyzer (Siemens Healthcare Diagnostics Ltd.).

### Liver biochemical parameters

#### *Determination of Superoxide Dismutase (SOD)*

The method by Muradian et al. (23) was adopted for the determination of SOD, which operates by detecting $O_2^{-}$ by oxidation of hydroxylamine HCl to 2 nitrites. The measurement of the colored product resulted at 560 nm calorimetrically. 100 µL of 5% liver homogenate in 0.2 M sucrose was included in 1 mL of sodium carbonate, 0.4 mL of nitro blue tetrazolium (NBT) and 0.2 ml of EDTA and zero minute reading was determined at 560 nm. 0.4 ml of hydroxylamine (1 mM) was included in the reaction mixture, following the incubation for 5 min at 25°C, the colored product acquired was measured to be at 560 nm colorimetrically. SOD activity was determined in units/mg protein as the quantity of protein in 1000 µL of 5% liver homogenate that prevented the reduction of 24 mM NBT by 50%.

#### *Determination of Catalase (CAT)*

The method by Sinha (1972) was adopted in order to determine the decomposition of hydrogen peroxide by tissue catalase(24). 100 µL of 5% liver homogenate in 0.15 M KCl was included in 1.9 mL of phosphate buffer (0.25 M, pH 7) and the absorbance level was measured to be 240 nm. 1 mL of hydrogen peroxide solution (0.34 mL of 30% $H_2O_2$ in 100 mL distilled water) was included in the 2:2 reaction mixture and the absorbance level was determined after 1 min at 240 nm. The activity of catalase was indicated as U/mg of protein. 1 international unit of CAT equals to the amount that catalyzes 1 mM of $H_2O_2$ per minute at 37°C.

#### *Determination of Glutathione Peroxidase (GPx)*

Method of Alexander (1962) was followed for estimation of glutathione peroxidase (GPx) from tissue homogenate (25). 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 KCI solution and 1 mL of sodium acetate (0.1M, pH5.25) and absorbance was measured colorimetrically 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. The activity of GPx was expressed as nanomoles of glutathione oxidized per minute per milligram protein.

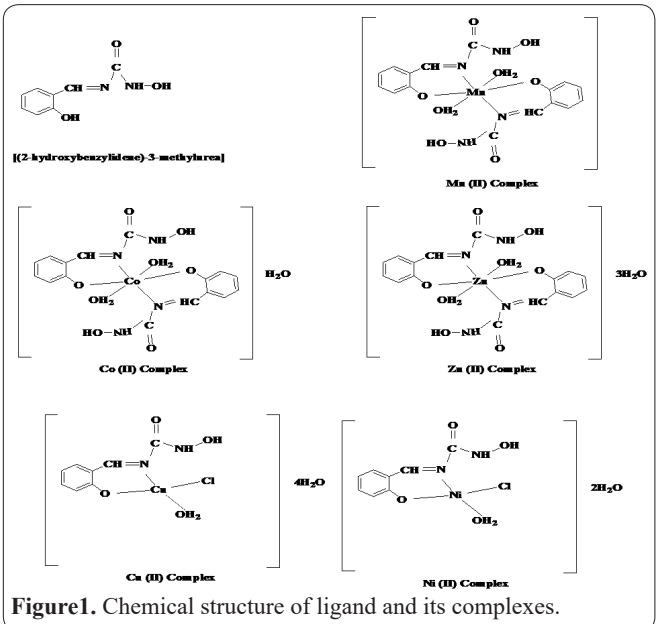


**Figure1.** Chemical structure of ligand and its complexes.

***Determination of Lipid Peroxidation***

Lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) was estimated as per the method of Fraga et al.,(1988) 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) (26). 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 programby using the mean ± standard derivation (SD). The results were analyzed for statistical significance by one-way ANOVA followed by Duncan's post hoctest of significance. $P < 0.05$ was considered as statistically significant.

## Results

Serum biochemical parameters (urea, creatine, total bilirubin, AST, ALT, and LDH) of rats in terms of Schiff bases derived from thiadiazole complexes were practiced and the rats of the control group were demonstrated in Table I. It was observed that ALT and LDH levels were increased on a small scale (P<0.05) in all groups compared to control group. Nevertheless, no significant difference was observed at levels of AST, creatine, urea and total bilirubin in all groups compared to control group (P<0.05). ALT level was determined to be higher (P<0.05) in the Cu-L group compared to other groups. Additionally, LDH level was determined to be higher (P<0.05) in the Cu-L and Co-L groups compared to other groups.

Liver biochemical parameters (SOD, MDA, CAT, GSH-Px) of rats in terms of Schiff bases derived from thiadiazole complexes were practiced and rats of the control group were demonstrated in Table II. SOD and MDA levels were observed to be increased moderately (P<0.05) in all groups compared to control group. Nonetheless, no significant difference was observed at levels of GSH-Px and CAT in all groups compared to control group (P>0.05). SOD level was determined to be higher (P<0.05) in the Cu-L, Mn-L and Zn-L groups compared to other groups. In addition, MDA level was observed to be higher (P<0.05) in the Ni-L, Cu-L, Zn-L groups compared to other groups.

## Discussion

Karatas et al. (2009) determined the effects of thiosemicarbazone derivatives on several blood parameters in rats. Significant differences were reported to exist (P<0.05) at the level of total bilirubin and the activities of AST and LDH in thiosemicarbazone [4-(1- phenyl-1-methyl cyclobutyl-3-yl)-2-(2-hydroxy benzylidene hydrazino) thiazole] group and activities of AST, GGT, LDH in thiosemicarbazone - zinc complex group compared to control group(27).

Yousef and Saddiq et al. (2012) prepared, characterized and investigated several microbiological, biochemical and histological parameters of pendant coumarin thiocarbohydrazone and its cobalt (II) complex in rats. In their study, the rats were divided into seven groups. Group (A), was a control group in which rats were administered ethanol 25 mg/kg. Group (B) was a control group in which the rats were administered ethanol 5 mg/kg. In Group (C), the rats were administered complex ($CoLNO_3.2H_2O$) 25 mg/kg. In Group (D), the rats were administered ligand (HL) 25 mg/kg. In Group (E), the rats were given complex ($CoLNO_3.2H_2O$) 5 mg/kg. In Group (F), the rats were given ligand (HL) 5 mg/kg. In Group (C), the results indicated that there was a rather significant (P<0.001) decrease in the activity of AST after 6 and 12 days, and a decrease in the activity of ALT after 12 days followingthe first 6 days (28).

The antitumor activities of $Mn^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Cu^{2+}$ chelates of anthracene-9- carboxaldehyde thiosemicarbazone (29) and the cytotoxic activities of phenylglyoxal bis(thiosemicarbazone) against *Ehrlich ascites* carcinoma cells were reported to exist. Additionally, these compounds were examined for antimicrobial activities on *B.subtilis* and *E.coli,* and it was determined that they prevented the bacterial growth at a considerable level (30).

Sari and Cukurovali et al. reported a new synthesized compound thiosemicarbazone thiazole ring containing a Schiff base and investigated the effect of 1-(1-mesityl-1-methylcyclobutane-3-yl)-2-suksinimido etanon thiosemicarbazone (MSTSC) the levels of malondialdehyde (MDA) in serum, liver, and kidney of rabbits. In the study, a statistically significant difference was not observed among control group MDA levels and liver MDA levels at the end of 2 and 8 days while a statistically significant difference was observed at the end of 16 and 60 days (P< 0,05). This state indicates a decrease in lipid peroxidation level at the end of 16 and 60 days in the liver. A statistically significant difference was not observed among control group MDA levels and Serum MDA levels. Likewise, no statistically significant difference was observed among control group MDA levels and kidney MDA levels (31).

CAT, SOD, GR and GPX are instances of enzymatic antioxidants. SOD and CAT are recognized as primary enzymes because they play a role in the direct elimination of reactive oxygen species. SOD is a significant defense enzyme, which catalyzes the dismutation of superoxide radicals and suggested to be the first enzyme which demonstrates a response against oxygen radicals (32). CAT is recognized as a hemoprotein, which catalyzes the reduction of hydrogen peroxides and defends tissues from highly reactive hydroxyl radicals. SOD and CAT exist in all oxygen-metabolizing cells and they act as a defense against potentially damaging reactivities of superoxide and hydrogen peroxide (33).

In study of Karagozoglu et al. (2013), 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, 25mg/kg thiadiazole-manganese, 25 mg/kg thiadiazole-cadmium and 25 mg/kg thiadiazole-chromecomplexes was injected to the experimental groups rats subcutaneously for 15days with three-day interval during the test.According to the result, the level of AST wasfound to be lower (P<0.05) in the Cr-L group than in other groups. The levels of ALT andtotal bilirubine were found to be lower (P<0.05) in the

**Table 1.** Effects of the Hydroxyurea Derivative Schiff Bases on Serum Biochemical Parameters in Rats.

| Parameters (n=7) | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Control*** | ***Ligand*** | ***Mn(L)$_2$*** | ***Cu(L)$_2$*** | ***Ni(L)$_2$*** | ***Co(L)$_2$*** | ***Zn(L)$_2$*** | **P** |
| AST | 158.50±2.72 | 142.75±5.18 | 161.50±4.93 | 168.75±2.21 | 191.50±6.46 | 179.25±4.78 | 184.25±3.50 | (P>0.05) |
| ALT | 49.25±1.70 | 37.00±2.16 $^{d}$ | 83.50±6.85$^{b}$ | 90.25±4.03 $^{a}$ | 81.00±2.58 $^{b}$ | 74.25±3.86 $^{c}$ | 82.75±0.95 $^{b}$ | **(P<0.05)** |
| LDH | 1116.00±5.88 | 1236.25±20.79$^{f}$ | 1461.75±26.63$^{e}$ | 1933.50±22.72$^{a}$ | 1701.50±51.00$^{c}$ | 1862.50±9.82$^{b}$ | 1603.00±43.58$^{d}$ | **(P<0.05)** |
| Creatinine | 0.71±0.03 | 0.61±0.04 | 0.43±0.03 | 0.22±0.02 | 0.23±0.05 | 0.31±0.05 | 0.52±0.03 | (P>0.05) |
| Urea | 70.50±8.18 | 62.50±4.65 | 58.75±3.30 | 39.50±3.87 | 33.75±4.99 | 54.75±4.03 | 45.50±5.44 | (P>0.05) |
| Total Bilirubine | 0.35±0.03 | 0.25±0.02 | 0.26±0.03 | 0.18±0.03 | 0.24±0.02 | 0.17±0.02 | 0.28±0.03 | (P>0.05) |

Mean ± Standard Deviation(SD). Each mean represents analyses of five independent samples(a, b, c, d, e, f) 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 2.** Effects of the Hydroxyurea Derivative Schiff Bases on Liver Biochemical Parameters in Rats.

| Parameters (n =7) | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Control*** | ***Ligand*** | ***Mn(L)$_2$*** | ***Cu(L)$_2$*** | ***Ni(L)$_2$*** | ***Co(L)$_2$*** | ***Zn(L)$_2$*** | **P** |
| SOD | 50.89±1.70 | 56.76±0.61 $^{f}$ | 74.13±0.64 $^{b}$ | 84.23±0.65$^{a}$ | 62.39±0.67 $^{d}$ | 59.27±1.19 $^{e}$ | 67.62±0.46$^{c}$ | **(P<0.05)** |
| LPO | 6.40±0.36 | 9.32±0.11 $^{e}$ | 8.32±0.17 $^{f}$ | 11.61±1.70 $^{b}$ | 14.02±0.29 $^{a}$ | 10.57±0.31 $^{d}$ | 11.31±0.24 $^{bcd}$ | **(P<0.05)** |
| CAT | 39.77±0.26 | 35.20±0.37 | 23.87±0.22 | 26.20±0.42 | 32.97±0.20 | 37.01±0.43 | 29.08±0.45 | (P>0.05) |
| GPX | 4.64±0.05 | 4.22±0.04 | 4.30±0.03 | 3.45±0.03 | 3.13±0.04 | 4.01±0.10 | 3.83±0.06 | (P>0.05) |

Mean ± Standard Deviation(SD), each mean represents analyses of five independent samples (a, b, c, d, e, f) 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. Values expressed: LPO, nmol malondialdehyde released/mg protein; GPx, nmol GSH oxidized $min^{-1}$ $mg^{-1}$ protein; CAT, nmol $H_2O_2$ decomposed $min^{-1}$ $mg^{-1}$ protein; SOD, activity was determined in units/mg protein as the quantity of protein in 1000 µL of 5% liver homogenate that prevented the reduction of 24 mM NBT by 50%.

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 (34).

Parlak et al. (2013) reported the effects of Mannich bases containing bis-1,2,4-triazole on the levels of *in vivo* malondialdehyde (MDA) in serum, livers and kidneys of rats. Statistically significant differences were emerged in all other test compounds of 50 μM and 100μM concentrations compared to control, except for the L3 compound. The compounds L1 and L4 significantly decreased the level of MDA. The compound L2 significantly was increased the level of MDA when compared to the control group. For the compound of L3, although the difference was not found highly significant, a decrease in MDA levels was observed in comparison to the control. Thus, the compound L2 was showed prooxidant effect by increasing the MDA level relative to other compounds (35).

It was determined that hydroxyurea derivative Schiff bases in rat liver tissue caused oxidative stress depending on Schiff bases. As a result, MDA, which was the last product of lipid peroxidation, increased, and in order to prevent the damage from SOD's activity, one of the antioxidant enzymes increased. In contrast to SOD, the reason of the decrease in GPx and CAT can be the direct inhibition of these enzymes of Schiff bases compounds. However, much more superoxide (The increase in SOD activity and MDA level is its indicator) can prevent CAT and GPx.

The elevation in AST, ALT and LDH enzyme activities indicated that it caused liver damage. Alternatively, it can be suggested that the utilized compounds led to toxic effects and it caused a protective effect in liver toxicity.

Consequently, it can be suggested that as hydroxyurea derivative Schiff bases demonstrate antioxidant and hepatotoxic activities and their pharmacological properties will be beneficial in numerous fields of application.

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