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THE EFFECTS HYDROXYUREA DERİVATİVE SCHİFF BASES ON FATTY ACİDS AND LYPOPHİLİC VİTAMİNS IN LIVER OF RATS

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Schiff bases and their metal complexes have antiviral, antimicrobial, antitumoral, radical scavenging and antioxidant activities. In this study, effects of Schiff base ligand and ıts metal complexes on the fatty acids and lyphophlilic vitamins of the liver of rats were investigated. The fatty acids of the liver were determined by GC and lyphophlilic vitamins of the liver determined by HPLC. The amounts of stearic acid (18:0), oleic acid (18:1) and monounsaturated fatty acids (MUFA) considerably decreased in the Mn, Ni and Zn complexes groups, whereas the amount of linolenic acid (18:3) and eicosatrienoic acid (20:3) considerably increased in the Ni an Zn complexes groups compared to the control group ($P<0.01$).However, there was no significant difference ($P>0.05$) in the amounts of arachidonic acid (20:4), docosahexaenoic acid (22:6), total saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), total omega-3 and omega- 6 fatty acids between groups. The amounts of vitamin K_1, vitamin D_2 considerably increased ($P<0.05$) in the Mn and Ni complexes groups as compared to the control. It was observed that the amount of α-tocopherol increased both in the Ni and Co complexes groups when compared to the control group but this increase was parallel to the amount in the same groups ($P<0.05$). As a result, it can be think that with the application of toxic metals such as manganese, cupper, nickel, cobalt, and zinc unsaturated fatty acids affected the activities of enzymes which are in the liver tissue and in charge in the fatty acid chain elongation.

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1. Introduction

Schiff bases and their metalcomplexes are used therapeutically in various diseases due to the properties such as antitumoral [1], antiviral [2], antimicrobial [3].In addition to this, it is reportted that Schiff bases and copper (II) complexes show antioxidativeactivity by inbiting lipid peroxidation [4]. Four coordinated Co (II) Schiff base chelate complexes show cataltic activity in oxygenation of alkene [5] and synthetic iron (II) Schiff base complex exhibits catalytic activity towards electro-reduction of oxygen [6]. Several Schiff base possess anti-inflammatory, allergic inhibitors reducing activity and radical scavenging [7], analgesic [8] and anti-oxidative action [9].

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Schiff bases derived from thiazole derivatives are reported to have significant anticancer activity[10]. Many of the anticancer drugs are viable ligands [11]. Some of these drugs exhibit increased anticancer activity when administered as metal complexes [12, 13]. Physical characteristics and antifungal activity of several 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 extensively studied [14, 15]. Sixteen Schiff base complexes of Co, Ni, Cu, and Zn derived from substituted thiazoles and substituted salicylaldehydes were prepared and tested for their antineoplastic potency against L-1210 lymphoid leukemia [16].

In a study that Karatepe and Karatas, the rats were injected subcutaneously with a new thiosemicarbazone (HL) and its CuL_2 and ZnL_2 complexes. The aim of their study was to determine the effect of the new compounds on the serum antioxidant vitamins (A, E, C), selenium (Se), malondialdehyde (MDA) levels, erythrocyte GSH-Px enzyme activity and morphological changes in the liver, kidney and adrenal gland tissues. It was observed that erythrocyte GSH-Px activity, serum MDA and vitamins A, E concentrations were statistically changed, but serum levels of selenium, and vitamin C were not changed. In conclusion, the parameters measured show that CuL_2 caused considerable oxidative stress and ZnL_2 behaved as an antioxidant [17].

The aim of the study was to assess the fatty acids and lypophilic vitamins (A,D,E and K vitamins) in the liver of experimental and control group rats and compare these with different groups such as ligand, Schiff base-manganese, Schiff base-cobalt, Schiff base-zinc, Schiff base-copper, Schiff base-nickel according to the control.

2. Materials and Methods

2.1 Animals

In the study 49 adult male wistar rats, raised inFırat 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. Hydroxyurea Schiff base complexes were diluted with corn oil in a way that its amount would be below % 10 as dimethylsulfoxide (DMSO) also dissolved [18]. Animals were divided into 1 control group and 6 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 wasinjected subcutaneously to ligand and the other metal complex groups for 15 days with three-day interval during the test [19].

2.2 Chemicals

Hydroxyurea derivative Schiff base compounds and their metal complexes used in the applications were synthesized and characterized by Sekerci et al [20, 21, 22]. The structure of ligands and their complexes are below (Fig. 1).

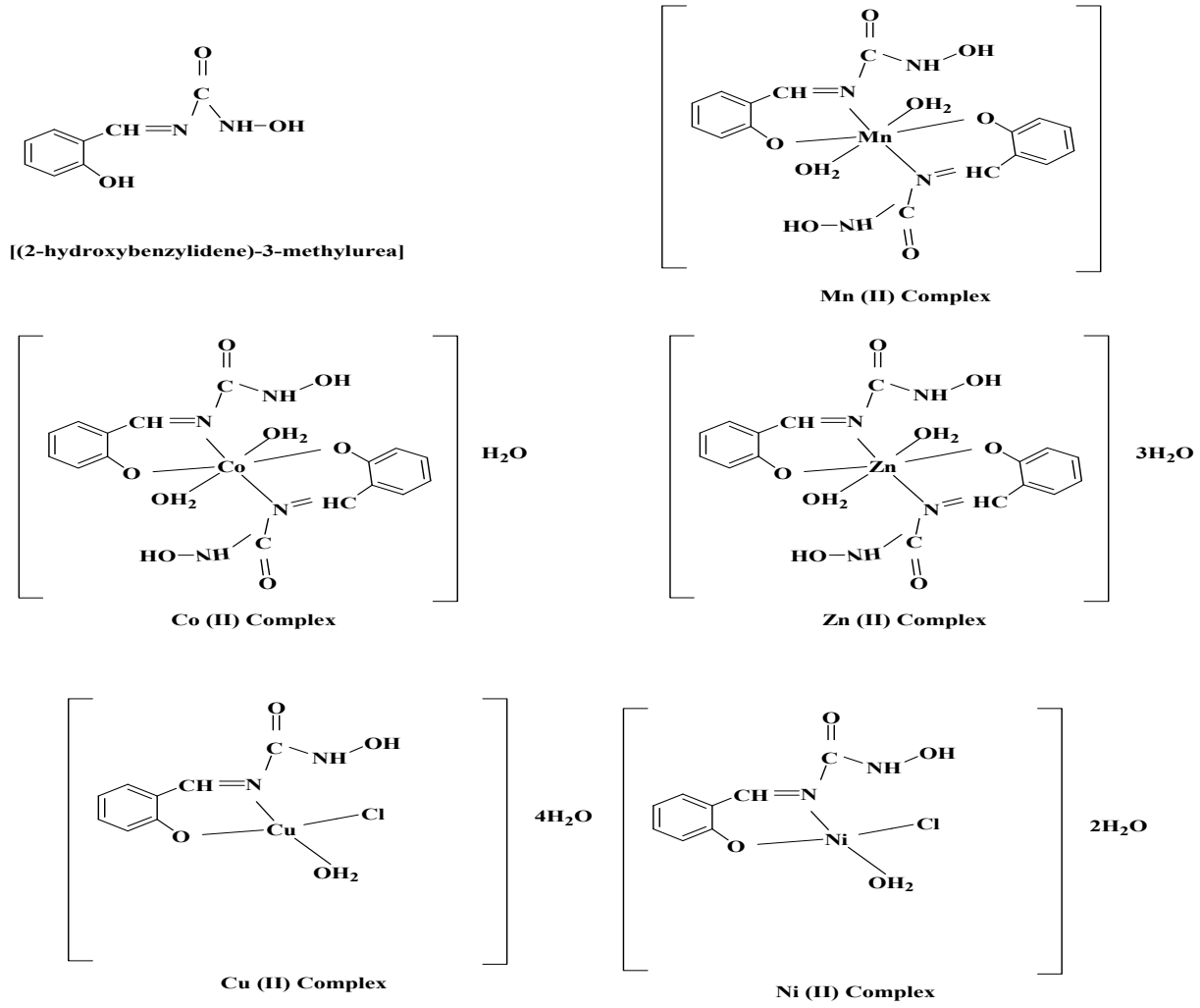

Fig. 1. Chemical structure of ligand and ıts complexes

2.3 Extraction of lipids

Liver tissue samples were taken and homogenized with a mixer. Three grams of homogenized liver tissue samples were taken and mixed with chloroform-methanol (2:1,v/v) in a mixer. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C and the supernatant part was used in the ADEK vitamin and fatty acid analysis [23]. Nonlipid contaminants in lipid extracts were removed by 0.88% KCl solution. The extracts were evaporated in rotary evaporator flask and then stored at -25°C.

2.4 Preparation of fatty acid methyl esters

An aliquot was taken from the supernatant part of the liver tissue and 5 mL of 2% methanolic sulphuric acid was added. The mixture was vortexed and then kept at 50°C for 12 h. Then, after being cooled to room temperature, 5 mL of 5% sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with 2×5 mL hexane. Fatty acid methyl esters were treated with 5 mL 2% $KHCO_3$ solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 1 mL fresh hexane [24] they were taken to auto sampler vials.

2.5 Gas chromatographic analysis of fatty acid methyl esters

Methyl esters were analyzed with the SHIMADZU GC 17 Ver. 3 gas chromatography (Kyoto, Japan). For this analysis, 25 m of long Machery-Nagel (Germany) capillary colon with an inner diameter of 0,25 μm and a thickness of 25 micron film was used. During the analysis, the

colon temperature was kept at 120-220°C, injection temperature was kept at 240°C and the detector temperature was kept at 280°C. The colon temperature program was adjusted from 120-220°C and the temperature increase was determined to be 5°C min^{-1} until 200 and 4°C min^{-1} from 200-220°C. It was kept at 220°C for 8 min and the total duration was set as 35 min and nitrogen gas was used as the carrier gas. During the analysis, before the analysis of fatty acid methyl esters, mixtures of standard fatty acid methyl esters were injected and the residence time of each fatty acid was determined. After this process, the necessary programming was made and the fatty acid methyl esters mixtures of the samples were analyzed [24].

2.6. HPLC analysis of ADEK vitamins and sterol amount

The 5 mL supernatant was taken to 25 mL tubes with caps and 5% KOH solution was added. After it was vortexed, it was kept at 85°C for 15 min. The tubes were then taken and cooled to room temperature and 5 mL of pure water was added and mixed. Lypophlic molecules that did not saponify were extracted with 2×5 mL hexane. The Hexane phase was evaporated with nitrogen flow. It was dissolved in 1 mL (50 + 50%, v v^{-1}) acetonitril/methanol mixture and then was taken to auto sampler vials and was analyzed.

The analysis was made with the Shimadzu brand HPLC device. In the device as the pump LC-10 ADVP UVvisible, as the detector SPD-10AVP, as column oven CTO-10ASVP, as auto sampler SIL-10ADVP, as degasser unit DGU-14A and Class VP software (Shimadzu, Kyoto Japan) was used and during the mobile phase the acetonitril/ methanol (60+40% v v^{-1}) mixture was used. The mobile phase flow rate was determined to be 1 mL A UV detector was used for the analysis and as a column the Supelcosil LC 18 (15×4.6 cm, 5 μm; Sigma,USA) column was used. For vitamin A and beta-charoten, detection of wave length 326 nm, for vitamin E, 202 nm and for vitamin D and K, 265 nm was used [25].

2.7. Statistical analysis

For statistical analysis the SPSS 15.0 software program was used. The comparison between experimental groups and the control group was made using ANOVA and LSD tests.

3. Results

3.1. Fatty acids

In liver tissues, the amounts of palmitic acid (16:0), palmitoleic acid (16:1, n-7), significantly increased ($p<0.05$, $p< 0.001$) in the Mn, Cu, Ni and Zn complexes groups, whereas the level of these fatty acids decreased ($p<0.01$) in the Co complex groups when compared to the control group. The amounts of stearic acid (18:0), oleic acid (18:1, n-9) and monounsaturated fatty acids (MUFA)considerably decreasedin the Mn, Ni and Zn complexes groups, whereas the amount of linolenic acid (18:3, n-3) and eicosatrienoic acid (20:3, n-6) considerably increasedin the Ni an Zn complexes groups compared to the control group($p<0.01$).However, there was no significant difference ($p>0.05$) in the amounts of arachidonic acid (20:4, n-6), docosahexaenoic acid (22:6,n-3), total saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), total omega-3 and omega- 6 fatty acids between groups.While the amount of linoleic acid (18:2, n-6) was found to be lower ($p< 0.05$) in the ligand group than in other groups, the amount of eicosatrienoic acid (20:3, n-6) was found to be lower ($p < 0.05$) in the Mn complex group than in other groups (Table I).

Table I. Amounts of Fatty Acids in Liver Tissues

Fatty Acids	Control	Ligand	Mn Complex	Cu Complex	Ni Complex	Co Complex	Zn Complex
16:0	19,19± 0,76	18,55± 0,38[a]	19,83± 0,90[a]	19,61± 0,18[a]	20,52± 0,54[a]	18,23± 0,31[a]	20,58± 0,34[a]
16:1 n-7	1,55± 0,09	2,17± 0,21[b]	3,80± 0,89[b]	2,12± 0,17[b]	2,49± 0,44[b]	1,27± 0,02[b]	1,68± 0,05[b]
18:0	18,36± 0,52	18,4± 0,66[a]	17,27± 0,6[a]	17,89± 0,7[a]	17,77± 0,48[a]	16,09± 0,41[a]	16,45± 0,33[a]
18:1 n-9	9,03± 0,61	4,10± 0,17[c]	8,98± 0,57[c]	8,99± 0,89[c]	7,91± 0,24[c]	10,31± 1,33[c]	8,06± 0,65[c]
18:2 n-6	18,03± 0,14	16,51± 1,72[a]	16,96± 0,63[a]	15,48± 1,49[a]	18,27± 0,80[a]	21,75± 1,50[a]	18,90± 0,40[a]
18:3 n-3	0,19± 0,03	0,27± 0,05[b]	0,18± 0,03[b]	0,42± 0,11[b]	0,10± 0,00[b]	0,37±0,12[b]	0,10± 0,01[b]
20:3 n-6	0,82± 0,02	1,38± 0,20[c]	0,88± 0,05[c]	0,93± 0,04[c]	0,10± 0,01[c]	0,55± 0,02[c]	0,70± 0,04[c]
20:4 n-6	27,05± 0,94	26,63± 1,05	26,89± 0,72	24,36± 1,58	27,77± 0,87	24,69± 1,64	27,16± 0,67
22:2	0,68± 0,03	0,60± 0,05[c]	0,36± 0,08[c]	0,70± 0,03[c]	0,10± 0,01[c]	0,57± 0,05[c]	0,30± 0,11[c]
22:5 n-3	1,28± 0,08	0,95± 0,08[b]	0,94± 0,11[b]	1,47± 0,20[b]	1,15± 0,06[b]	1,25± 0,05[b]	0,85± 0,09[b]
22:6 n-3	2,97± 0,03	3,32± 0,40	3,28± 0,48	1,86± 0,72	3,14± 0,05	2,87± 0,30	3,62± 0,31
ΣSFA	37,55± 0,64	37,00± 0,52	37,10±0,75	37,50± 0,45	38,30± 0,51	34,32± 0,35[a]	37,03± 0,34
ΣMUFA	10,58± 0,35	6,27±0,20	12,78±0,73	11,11±0,58	10,40±0,34	11,58± 0,68	9,74± 0,35
ΣPUFA	51,02± 0,18	54,66± 0,57	49,49± 0,30	45,22± 0,60	50,63± 0,25	52,05± 0,51	51,63± 0,23
Σ w-3	4,44 ± 0,04	4,54±0,17	4,40±0,2	3,75±0,34	4,39±0,03	4,49± 0,15	4,57± 0,13
Σ w-6	45,90±0,36	44,52±0,99	44,73±0,46	40,77±1,03	46,14±0,56	46,99± 1,05	46,76± 0,37

a: $p<0.05$, b: $p<0.01$, c: $p<0.001$

3.2. Lypophilic vitamins

Therewas no significant difference ($p>0.05$) in the amount of cholesterol and K_2 and retinol vitamin between groups. The amounts of vitamin K_1, vitamin D_2 considerably increased ($p< 0.05$) in the Mn and Ni complexes groups as compared to the control. It was observed that the amount of α-tocopherol increased both in the Ni and Co complexes groups when compared to the control group but this increase was parallel to the amount in the same groups ($p<0.05$). It was observed that the amount of D_3 decreased in the Ni Co and Zn complexes groups when compared to the control group but this decrease was parallel to the amount in the same groups ($p<0.05$) The amount of vitamin K_1 were found to be lower ($p< 0.05$) in the ligand, Cu complex groups than in other groups. On the otherhand the amount of α-tocopherol were found to be lower ($p< 0.05$) in the ligand, Mn and Zn complexes groups than in other groups. As for the ergesterol amount, it was observed that this amount decreased ($p<0.05$) in the Cu, Ni and Co complexes groups than in other groups (Table II).

Table II. Amounts of Lypophilic Vitamins in Liver Tissues

Vitamins	Control	Ligand	Mn Complex	Cu Complex	Ni Complex	Co Complex	Zn Complex
Cholesterol	1066,5±124,1	807,3±105,2	834,86±88,1	326,2±146,8	710,9±133,4	937,3±60,08	1108,4±13
K1	2,45± 0,29	1,23± 0,11[a]	3,28± 0,21[a]	1,40± 0,63[a]	2,60± 0,85[a]	2,50± 0,12[a]	3,13± 0,38[a]
K2	3,84± 0,4	4,56± 0,26	5,41± 0,78	0,88± 0,83	1,98±0,98	4,55± 0,37	4,40± 0,77
D2	0,26± 0,21	0,13± 0,06[a]	0,28± 0,10[a]	0,28± 0,15[a]	0,33± 0,06[a]	0,12± 0,05[a]	0,15± 0,07[a]
D3	0,37± 0,14	1,36± 1,09[c]	0,58± 0,08[c]	0,10± 0,05[c]	0,23± 0,06[c]	0,23± 0,03[c]	0,23± 0,04[c]
α-tocopherol	10,89± 0,58	14,5± 3,65[c]	12,53± 0,72[c]	5,01± 2,34[c]	10,1± 1,19[c]	10,31±0,51[c]	13,53±3,41[c]
Retinol	346,74±8,84	246,8±37,1	459,9±11,8	204,95±106	327,95±64	334,76±22,6	334,8±22,6
Ergosterol	3,84± 0,42	3,78±1,75[c]	7,75± 3,48[c]	1,36± 0,51[c]	2,16± 0,37[c]	2,35± 0,24[c]	3,28±0,40[c]

a: $p<0.05$, b: $p<0.01$, c: $p<0.001$

4. Discussion

According of our study results, the amount of linoleic acid in the liver of Schiff base-nickel, Schiff bas-cobalt and Schiff base-zinc complexes groups increased comparison to the control group. In a previously conducted study it is stated that Δ-9 the activityof desaturase is inhibited with the application of cadmium [26]. In the study of Dayangaç et al. (2006) Investigated effects of cadmium on fatty acids composition in some tissues (liver, kidney, testis and heart tissues) of rats and it was observed that while palmitic acid (C16:0) and stearic acid (C18:0) amounts of cadmium group decreased, oleic acid (C18:1, n-9), linoleic acid (18:2, n-6) arachidonic (C20:4, n-6) acid docosahexaenoic acid (22:6, n-3) amounts increased acccording to control group in liver tissues. Oleic acid (C18:1n9) and stearic acid (C18:0) amounts of cadmium group increased acccording to control group in kidney tissues ($P<0.05$). While oleic acid values of cadmium group in testis tissues decreased, arachidonic (C20:4n6) acid values increased [27].

Long-chain unsaturated fatty acids are sensitive to the disruptive impact of the molecular oxygen or peroxide. Linoleic, linolenic and arachidonic acids, which are unsaturated fatty acids, are named as essential fatty acids and they are significant in the metabolism of the organism. It is stated that the increase in lipid peroxidation in tissues stemmed from the increase in catalytic peroxidation of linoleic acid by means of various metal ions [28]. In the other study of Sarı and Çukurovalı et al. (2008) investigated the effects of thiosemicarbazone derivatives l-(lmesityl- l-methylcylobutane-3-yl)-2-succinoimido ethanone thiosemicarbazone (MSTSC) and l-(l-phenyl-lmethylcylobutane- 3-yl)-2-succinoimido ethanone thiosemicarbazone (FSTSC) materials on the fatty acids of the liver of rabbits. And it was reported that while there were decreased palmitic acid (C16:0), stearic acid (C18:0) of MSTSC and FSTSC groups ($p<0,01$), there was increased fatty acid level of linoleic acid (C18:2) and oleic acid (C18:1) compared to the control group [29].

It has determined that linoleic acid (18:2 n-6) and arachidonic acid (20:4) decreased specially in the out of control groups. Arachidonic acid is a fatty acid that is synthesized from the linoleic acid by the delta 6 desaturase way. Although the linoleic acid decreased, the quantity of arachidonic acid did not increase and this event showed that there were some other factors beside synthesis. Decreasing of both linoleic and arachidonic acid quantity can be resulted from eicosenoid biosynthesis increase. Specially decrease in arachidonic acid quantity can be related with eicosenoid's synthesis (20:1 n-9). Because these molecules called eicosenoid and effective

like hormone provide that arachidonic acid turns to leukotriens, prostaglandins and thromboxane being used by lipoxygenase and cyclooxygenase enzymes. The decrease of arachidonic acid can be explained by this methabolic way's being active.

In conclusion, it is thought that with the application of toxic metals such as manganese, cupper, nickel, cobalt, and zinc unsaturated fatty acids affected the activities of enzymes which are in the liver tissue and in charge in the fatty acid chain elongation.

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