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MDA Effect of the Thiosemicarbazone Derivative Schiff Base 1-(1-mesityl-1-methylcyclobutane-3-yl)-2-suksinimido Etanon Thiosemicarbazone in Rabbits

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Abstract. In this study, the rabbits were injected subcutaneously with a new thiosemicarbazone thiazole ring containing a Schiff base, 1-(1-mesityl-1-methylcyclobutane-3-yl)-2-suksinimido etanon thiosemicarbazone, (25 mg/ kg body weight), and then sacrificed after 2 th, 8 th, 16 th , 60 th days of being injected. The aim of this study was to determine the effect of the new compounds (on the serum, liver and kidney). MDA concentration was determined by HPLC with working on blood serum, liver and kidney. Experimental groups and control groups were compared to each other. The liver MDA concentrations changed statistically ($p<0.05$) while serum concentrations of MDA in the kidney was unchanged. As a result, these parameters measured in the control group showed effects in the liver MDA. In addition to the many pharmacological properties of derivatives of TSC surveyed believe that these parameters will contribute to the knowledge of the literature.

Keywords: Thiosemicarbazone, MDA, Rabbit, Serum, Liver, Kidney, HPLC.

1. Introduction

In drug and chemical research, one of the key issues is to create new and more effective cancer drugs and investigate the antitumor effects of cancer treatment uses [1]**.** It is known that Schiff base and it's derivatives containing thiosemicarbazone show antitumor and antifungal activities. Lipid peroxidation is a free radical chain reaction [5] which causes the degeneration of cell membranes. Most products of lipid peroxidation are known to have mutagenic and/or carcinogenic properties [6]. Free radical species affect all important components of cells such as lipids, proteins, carbohydrates and nucleic acids [7]. Lipids are oxidized by free radical attack, and hence membranes are damaged [8]. Lipid peroxides are disintegrated quickly and form reactive carbon compounds. MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation [9]. Byrnes et al., [10] reported that copper thiosemicarbazone complex caused oxidative stress by inducing free radical production. Kaur and Ali, [11] stated that 2-aryl-3-[4-(substitute feryl)-3-thiosemicarbazone] prevented lipid peroxidation in rat liver. Likewise, the thiosemicarbazone derivative caused a decrease in MDA level in diabetic rat liver cells [12].

In this study, the rabbits were injected subcutaneously with a new thiosemicarbazone thiazole ring containing a Schiff base, and then sacrificed after 2 th, 8 th, 16 th , 60 th days of being injected. And we investigated the levels of malondialdehyde (MDA) in rabbit's serum, liver and kidney.

2. Material and Methods

In the experimental application, 4-6 months male New Zealand white rabbits were used. All animals were subjected to a 10 days process of adaptation. The media was kept constant conditions of room temperature (24-26°C). Enough water and feed for animals fed (ad libitum) was given..

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In this study, a new synthesized compound was used. Substance used in this study, 1-(1-mesityl-1-methylcyclobutane-3-yl)-2-suksinimido etanon thiosemicarbazone (MSTSC) was synthesized by Cukurovali et al. [13].The chemical structure is shown below (Figure 1).

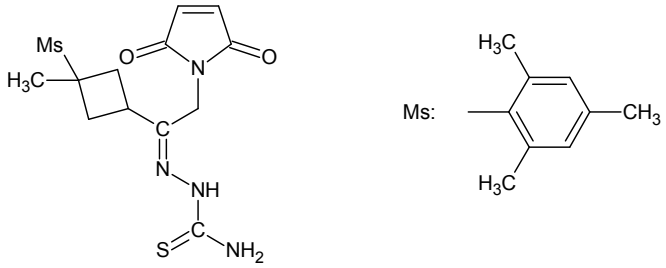

Figure1: 1-(1-mesityl-1- methylcyclobutane-3-yl)-2-suksinimido etanon thiosemicarbazone (MSTSC)

The animals were divided into two groups (control and MSTSC group). The control group was given only injection of 250 µl, 10% dimethylsulphoxide (DMSO) in corn oil the day after. The other group of rabbits was injected with substance MSTSC, substance (20 mg kg-1, dissolved 250 µl 10% DMSO in corn oil) [14]. Injections were continued for 2 day, 8 day, 16 day and 60 days. For subcutaneous treatment, stock solutions of the compounds were made immediately before use. They were suspended in corn oil at the desired concentration following initial solution in 10% DMSO. This concentration of DMSO by itself produced no observable toxic effects [15]. All animals were on a normal diet throughout the experimental period. After 2 days, after 8 day, after 16 day and after 60 days injections were sacrificed under ether anesthesia that obtained 5 rabbit from the each groups (control and administration of substance). Serum, liver and kidney samples were harvested and kept at –20ºC until analyzed.

2.1. Analysis

Serum samples were centrifuged at 3500 rpm for 5 minutes at 4°C and the liver and kidney tissue was separated. The extractions of free MDA were performed according to Cerhata et al. [16].The supernatant was filtered and was determined by using the method of Tavazzi et al. [17]. A Supelcosil LC-18-DB HPLC reversed-phase column (3 mm particle size and 250 x 3.9 ID) was utilized for the detection of MDA levels.

While a 3.7 mM phosphate buffer, pH 4.0 mobile phase was used at 1.0 mL min-1 flow rate to determine, the free MDA level was determined with a 30 mM KH_2PO_4 buffer, pH=4 with H_3PO_4 and methanol (65%-35% v/v) mobile phase at 1.5 mL min-1 flow rate. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemical Co.(Darmstadt, Germany). Bi-distilled water used to in the all studies.

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2.2. Statically Analysis

Data were reported as means SE Statistical analysis was performed using SPSS 10.0 software. Analysis of variance (Kruskal Wallis) and LSD test were used for comparison between groups.

3. Results

Parameters to the results of experimental studies with control groups in each of the groups throughout the application were given in the Tables 1-3. Serum, liver and kidney MDA levels were statistically significant differences. Effects of the compound on the serum were demonstrated in Table 1.

Table 1. Effects of the compounds on the serum

	Days			
Groups	2 (n=6)	8 (n=6)	16 (n=6)	60 (n=6)

MDA ($mg\,mL^{-1}$)	Control	2,96 ±0,28	2,96 ±0,28	2,42 ±0,10	2,47 ±0,11
	(MSTSC)	1,92 ± 0,10	2,54 ±0,35	2,35 ±0,12	2,02 ±0,10
p		-	-	-	-

- : not important. p values of Kruskal Wallis analysis of variance and Mann-Whitney U test was held according to the result. Values are mean ± SD expressed

Effects of the compounds on the kidney tissues and liver tissues were demonstrated in Table 2 and 3 respectively.

Table 2. Effects of the compounds on the liver

Groups		Days			
		2 (n=6)	8 (n=6)	16 (n=6)	60 (n=6)
MDA ($mg\,mL^{-1}$)	Control	9,59 ±0,56	9,06 ±0,21	13,89 [a] ±1,37	12,73 [a] ±1,93
	(MSTSC)	7,91 ±1,17	7,19 ±0,22	11,73 [a] ±0,60	11,98 [a] ±0,27
p		-	-	*	*

* p< 0,05 - : not important . p values of Kruskal Wallis analysis of variance and Mann-Whitney U test was held according to the result. Values are mean ± SD expressed

Statistically significant difference was not found between control group MDA levels and Serum MDA levels. Similarly statistically significant difference was not found between control group MDA levels and kidney MDA levels. However; statistically significant difference was found between control group MDA levels and liver MDA levels (p< 0,05).

Table 3. Effects of the compounds on the kidney

Groups		Days			
		2 (n=6)	8 (n=6)	16 (n=6)	60 (n=6)
MDA ($mg\,mL^{-1}$)	Control	29,93 ±1,04	28,78 ±2,53	37,70 ±1,39	26,90 ±1,47
	(MSTSC)	28,53 ±2,19	25,49 ±2,20	31,96 ±2,89	24,51 ±1,34
	p	-	-	-	-

- : not important .p values of Kruskal Wallis analysis of variance and Mann-Whitney U test was held according to the result. Values are mean ± SD expressed

4. Discussion

Lipid peroxides are disintegrated quickly and form reactive carbon compounds. MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation [9].

Lipid peroxides formed in this way easily demolished and most importantly, malondialdehyde (MDA), which constitute the reactive carbon compounds. Therefore, measurement of the amount of MDA reflects the degree of lipid peroxidation in tissues [18]. Hrnciarova and co-workers administrated thiosemicarbozane derivatives to diabetic rats and investigated the level of lipid peoxidation. In the results of this survey, they obtained that an decreasing of level of lipid peroxidation [12]. As shown in Table 2, statistically significant difference was not found between control group MDA levels and liver MDA levels in end of 2 and 8 days while statistically significant difference was found in end of 16 and 60 days (p< 0,05). This situation show decreasing in lipid peroxidation level in end of 16 and 60 days in the liver. Statistically significant difference

was not found between control group MDA levels and Serum MDA levels. Similarly statistically significant difference was not found between control group MDA levels and kidney MDA levels.

We hope that this original work is potentially a useful addition to the literature and can guide to similar works.

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