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EFFECTS OF FOOD PRESERVATIVE NATAMYCIN ON LIVER ENZYMES AND TOTAL PROTEIN IN *MUS MUSCULUS*

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Abstract

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Natamycin is a food preservative of which is used to inhibit yeast and fungi growth on cheese and sausages. In the present study, effects of natamycin on the levels of liver enzymes and total protein were investigated in mice by using serum enzyme activity assay. Natamycin was intraperitoneally injected to female and male mice at 200, 400and 800 mg/kg for 6, 12 and 24 hours. Blood samples were taken for determination of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, total protein and all of samples were performed by using a spectrophometer. The present results revealed a significant increase in the levels of ALT of female and male mice treated with different concentrations of natamycin when compared with the negative control. Furthermore, natamycin induced a significant decrease in serum LDH and ALP in female and male mice, respectively. In conclusion, natamycin may affect adversely and cause degenerative disorders in liver, and so it may alter levels of enzymes in liver being a vital organ.

*Key words: f*ood preservative, liver enzymes, mice, natamycin, total protein

Abbreviations: ALT - alanine aminotransferase; ALP - alkaline phosphatase; AST - aspartate aminotransferase; LDH - lactate dehydrogenase

Introduction

In recent years, the widespread use of food additives is a consequence of developing industry, increasing population and consumption of food. Therefore, it is essential to find new food sources and preserve them for a long time without molding. The great bulk of food additives used food is preservatives.

The food preservatives nip or slow the growing of microorganisms being pathogenic in humans as mold, yeast and bacteria. The food preservatives are consisting of antimicrobials inhibiting the growing of bacteria or fungi and antioxidants inhibiting the oxidation of constituent compounds of food. The extensive usage of the food preservatives causes serious problems of health (Sarikaya and Solak, 2003). Therefore, reporting on the effects of food preservatives by using different assays on the systems of living organism is important in metabolic and toxicological disorders studies.

Serum enzyme activity test in clinical studies is one of assays commonly used to determine functional disorders and test organ functions in mammalians. In recent years, these tests come into use by investigators, which generally want to research environment biology and effects of environmental pollutants onto living things (Mayer et al., 1992). In general, substances polluting cause the release of celluler enzymes by occurring tissue damage and so they induce the increasing in

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serum enzyme activities (Mayer et al., 1992). Enzymes are biologic catalysts and so they are crucial for metabolism. Aberant enzyme levels may be determinant of different diseases.

Natamycin is a food preservative of which is used to inhibit yeast and fungi growth on cheese and sausages (EMEA, 1998). In addition, natamycin is a polyene macrolide antibiotic produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. The activity of natamycin against yeasts and moulds, but not bacteria, is highly effective. It is used topically in veterinary medicine to treat mycotic infections, such as ringworm in cattle and horses. Previously, it was used topically against fungal infections of the skin and mucous membranes in humans. Its medical use is now confined to topical treatment of corneal fungal infections and the prevention of such infections in users of contact lens (WHO, 2006).

Some researchers (Hutchison et al., 1966; Levinskas et al., 1966; Wieriks, 1966, 1971 and Blankwater and Hespe, 1979.) studied the biochemical aspects of natamycin in rats, dogs and humans (WHO, 2006).There is available limited number of studies about toxic effects of natamycin on the liver enzymes. Further investigations are required to verify of this.

In this study, it is aimed to investigate the effect of natamycin which is used as food preservative on some biochemical parameters in serum of mice and whether natamycin cause damage on liver or not.

Materials and Methods

Natamycin (CAS No. 7681-93-8) (Figure 1) whose the commercial name is Delvocid was used as a test material and distilled water was used as negative control.

In our study, male and female mice *Mus musculus* (8–12 weeks of age) ranging in weight from 20-25 g were obtained from Trakya University Scientific Research Center. The animals were kept in closely inbred colony under conventional laboratory conditions at a room temperature of 25 ± 5°C and in 12 h dark and 12 h light cycles. Food pellets and water were provided *ad libitum*.

According to Van Eeken and Wubs (1976), the LD_{50} of natamycin (intraperitoneal) was found to be 1600 mg/kg bw (WHO, 2006).In the study, mice were injected with 200, 400 and 800 mg/kg bw (1/8, 1/4, 1/2 LD_{50}, respectively) concentrations of natamycin intraperitoneally.

In our study, 200, 400 and 800 mg/kg bw concentrations of natamycin were prepared in distilled water. In order to investigate effects of this food preservative on liver enzymes, ALT, AST, ALP, LDH levels were determined and total protein level also was observed.

Biochemical analysis

By the end of the experimental periods, blood samples, which obtained from heart were collected in dry glass centrifuge tubes and allowed to coagulate at room temperature and 3500 rpm for 15 min at room temperature for separation of serum. The serums were frozen at -20°C for biochemical analysis. Analysis of all samples were performed in a spectrophometer (Biosystems, BTS-310, Spain) by using commercial kits Diasis (Istanbul, Turkey) and enzyme levels were detected as Unit/Liter.

Statistical Analysis

The statistical analysis was carried out using Statistica Axa 7.1 program. Kalmogorov Smirnov test was used, and the significance between groups was determined using the one-way analysis of the variance (ANOVA), followed by a post hoc test. If ANOVA was significant, Dunnett's test was performed. The arithmetic mean ± SD (standard deviation) was evaluated as descriptive statistics, $P \leq 0.05$ was considered as the level of significance.

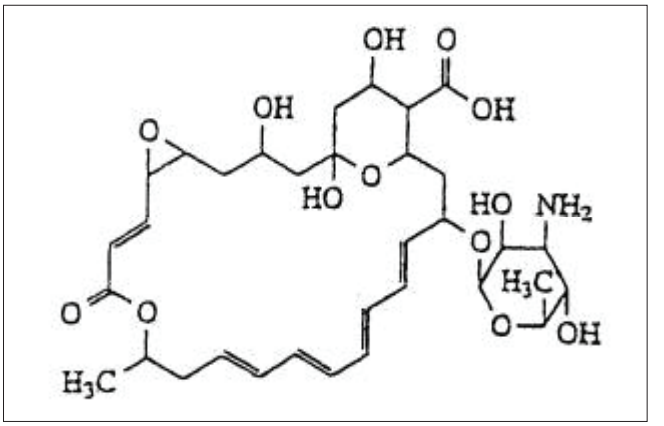

Fig. 1. Chemicalstructure of natamycin

Results

The results obtained are given in Tables 1 and 2. In female mice, a significant decrease was observed in LDH level at 800 mg/kg concentration of natamycin for 6, 12 and 24 hours when compared with the negative control. ALT activity significantly increased at 400 and 800 mg/kg concentrations of natamycin for 6h and 800 mg/kg concentration of natamycin for 12h. No significant alteration was observed in ALP, AST and total protein levels.

In male mice, ALP activity significantly decreased at all of concentrations of natamycin for 24 h. On the contrary, ALT activity significantly increased at 400 and 800 mg/kg concentrations of natamycin for 6 h. No significant alteration was determined in LDH, AST and total protein levels.

Table 1
Effects of natamycin on the serum liver enzymes and total protein levels in female mice

Treatment periods	Concentrations	Liver Enzymes and Total Protein (X±SE)				
		ALP	LDH	AST	ALT	Total Protein
6 hours	(-) control	436.07 + 45.66	2592.54 ± 125.82	169.43 ± 15.40	59.35 ± 10.72	5.16 ± 0.37
	200 mg/kg	408.97 ± 38.85	2172.62 ± 333.59	177.10 ± 11.45	111.04 ± 21.65	4.38 ± 0.16
	400 mg/kg	421.02 ± 45.82	2417.59 ± 415.44	181.46 ± 22.77	***180.83 ± 9.22	5.11 ± 0.07
	800 mg/kg	391.57 ± 7.77	*1212.04 ± 135.78	154.46 ± 26.33	***143.92 ± 13.93	4.83 ± 0.18
12 hours	(-) control	445.58 ± 58.09	3033.43 ± 105.62	290.73 ± 15.08	69.57 ± 6.43	5.00 ± 0.23
	200 mg/kg	258.67 ± 104.39	2705.05 ± 238.11	312.06 ± 33.63	173.05 ± 43.94	5.27 ± 0.09
	400 mg/kg	271.02 ± 68.57	2366.46 ± 396.12	250.40 ± 47.70	107.82 ± 12.68	4.43 ± 0.42
	800 mg/kg	444.37 ± 93.33	*1768.73 ± 239.72	125.03 ± 40.29	*234.77 ± 42.30	4.80 ± 0.16
24 hours	(-) control	375.74 ± 23.35	2514.74 ± 189.40	290.40 ± 4.06	124.27 ± 10.33	4.74 ± 0.12
	200 mg/kg	280.30 ± 51.27	2913.59 ± 369.57	263.97 ± 60.73	106.01 ± 13.61	4.57 ± 0.10
	400 mg/kg	284.03 ± 17.20	2585.70 ± 162.38	323.37 ± 14.04	110.83 ± 10.45	4.56 ± 0.07
	800 mg/kg	258.76 ± 24.60	*1429.07 ± 247.15	346.85 ± 11.70	96.17 ± 9.29	4.82 ± 0.03

*P<0.05; **P<0.01; ***P<0.001.

Table 2
Effects of natamycin on the serum liver enzymes and total protein levels in male mice

Treatment periods	Concentrations	Liver Enzymes and Total Protein (X±SE)				
		ALP	LDH	AST	ALT	Total Protein
6 hours	(-) control	284.78 ± 33.95	2865.24 ±107.30	163.28 ± 26.09	57.67 ± 4.65	4.27 ± 0.17
	200 mg/kg	436.50 ± 27.15	2648.62 ± 329.62	157.35 ± 31.52	51.70 ± 10.59	4.37 ± 0.17
	400 mg/kg	309.08 ± 52.10	2472.50 ± 174.32	231.68 ± 18.65	*108.82 ± 6.85	4.44 ± 0.15
	800 mg/kg	338.71 ± 57.94	2624.75 ± 285.65	140.25 ± 15.01	**132.85 ± 17.11	4.34 ± 0.06
12 hours	(-) control	313.26 ± 72.31	2613.40 ± 168.07	302.10 ± 40.24	183.78 ± 44.73	4.20 ± 0.19
	200 mg/kg	200.66 ± 25.44	2275.07 ± 186.59	304.63 ± 35.20	91.27 ± 10.76	4.47 ± 0.12
	400 mg/kg	309.08 ± 52.10	2472.50 ± 174.32	231.68 ± 18.65	108.82 ± 6.85	4.44 ± 0.15
	800 mg/kg	338.71 ± 57.94	2624.75 ± 285.65	140.25 ± 15.01	132.85 ± 17.11	4.34 ± 0.06
24 hours	(-) control	528.53 ± 68.92	2562.56 ± 191.02	348.83 ± 13.34	95.31 ± 3.22	4.20 ± 0.09
	200 mg/kg	**230.62 ± 29.37	2719.92 ± 184.53	289.17 ± 50.61	79.99 ± 2.94	4.55 ± 0.06
	400 mg/kg	**188.86 ± 3.76	2114.75 ± 295.42	345.00 ± 30.18	109.78 ± 17.41	4.59 ± 0.22
	800 mg/kg	*331.03 ± 50.37	2955.36 ± 139.85	348.97 ± 22.5	94.02 ± 13.03	4.23 ± 0.10

*P<0.05; **P<0.01; ***P<0.001.

Discussion

Liver is a target organ and primary site of detoxification, is generally the major site of intense metabolism, and is therefore prone to various disorders because of exposure to the toxins of extrinsic as well as intrinsic forms. Guyton and Hall (1996) stated that liver plays important role in metabolism to maintain energy level and structural stability of body. According to Hodgson (2004), it is also site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity. However, this will damage the liver cells and produce hepatotoxicity (Paliwal et al., 2009).

Serum aminotransferase activities are known as toxicity markers in the study of hepatotoxicity caused by chemicals (Govindwar and Dalvi, 1990). An increase in the activities of these enzymes is termed as the early recognition of toxic hepatitis (Al-Shinnawy, 2009).

The presents results indicated that the levels of ALT of female and male mice treated with different concentrations of natamycin recorded a marked increase throughout the treatment periods when compared with negative control. Furthermore, natamycin induced a significant decrease in serum LDH and ALP in female and male mice, respectively.

These results are in disagreement with Wieriks (1966, 1971) who observed that natamycin was not effective in rats fed with nutrition including natamycin (WHO, 2006).

Citric acid, sodium benzoate and sodium nitrite also are food preservatives. Aktac et al. (2003) reported that citric acid did not induce alterations in LDH, AST and ALT activities in mice. It was indicated that sodium benzoate increased AST and ALP levels in rats (Ibekwe et al., 2007) and sodium nitrite also cause a significant increase in ALT, AST, ALP and LDH activities (Helal and Elsaid, 2006). It is thought that these differences between the results may be related to food preservatives used differences in metabolisms of animals.

In our opinion, natamycin may cause degenerative disorders in liver. Alterations in serum levels of liver enzymes may ensue from release of enzymes into blood stream due to hepatic impairment.

According to Varely et al. (1988) the elevation of aminotransferases activities in serum may be due to tissue damage particularly in liver, kidney and heart. And increased permeability of cell membrane or increased synthesis or decreased catabolism of transaminases may be involved (Malik et al., 1980), also Westlake et al. (1981) mentioned that the release of abnormally high levels of specific tissue enzymes into blood stream is dependent on both the degree and the type of damage exerted by the toxic compound administration (Amin et al., 2010).

Webner (2003) reported that the damaged or diseased tissues release enzymes into the blood, so serum alkaline phosphatase measurements can be abnormal in many conditions including bone diseases and liver diseases and Shakoori et al. (1987) revealed that several enzymes like ALT, AST, LDH and ALP beach out into the serum and hence their level indicate the type and extent of damage inflicted (Amin et al., 2010).

Proteins are the fundamental components of all living cells and include many substances such as enzymes, hormones and antibodies. In this study, it was not observed effects of natamycin on the serum total protein levels in both female and male mice.

Conclusions

It is thought that natamycin may cause degenerative disorders in liver of mice due to alterations observed in serum levels of liver enzymes. It is necessary to carry out more investigations with regard to the general toxicity of natamycin in different organisms and assays. Furthermore, more extensive assessment of food preservatives commonly used is warranted.

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