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Effect of *Urtica dioica* L. on Selected Trace Elements Levels and Serum Protein Patterns in the Rabbits Treated with 7,12-dimethylbenz(a)anthracene

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In the present study, the effect of Nettle (*Urtica dioca* L.) on trace elements and serum protein patterns in female rabbits administrated to 7,12-Dimethylbenz(a)anthracene (DMBA) was investigated. Rabbits were divided into equal four groups. Group I was control group (n = 8), group II (n = 8), treated with DMBA (1 mg/kg.d), group III treated with DMBA (1 mg/kg.d) and methanol extract of *U. dioica* 35 mg/kg/d and group IV (n = 8) treated with DMBA (1 mg/kg.d) and aqueous extract of *U. dioica* 35 mg/kg/d. The results show that on 60th day DMBA-treated group were compared with the control group levels, a significant decrease ($p < 0.05$) was observed in the Zn. DMBA and methanol extract of *U. dioica* treated group were compared with the control group levels, a significant increase ($p < 0.05$) was observed in the Cu. Serum Fe and Mn levels among groups were determined not to be statistically significant ($p > 0.05$). It was found that *U. dioica* not affected on trace elements level and serum protein patterns in rabbits subjected to DMBA.

Key Words: 7,12-Dimethylbenz(a)anthracene, Trace elements, Serum proteins, *Urtica dioica* L.

INTRODUCTION

Most tumours that begins either the agent produces or is converted metabolically to the electrophilic reactants that binds covalently to cellular DNA. For several polycyclic aromatic hydrocarbons (PAH) such as 7,12-dimethylbenz(a)anthracene (DMBA), the ultimate carcinogen is called dihydrodiol epoxide of the Bay region, produced during cellular metabolism. Thus, free radicals and modified DNA bases have been implied strongly in the carcinogenesis in general[1].

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The formation of highly reactive oxygen-containing molecular species is a normal consequence of a variety of essential biochemical reactions. If a reactive molecule contains one or more of electrons unpaired, the molecule is termed a free radical[2]. The generation of highly reactive oxygen species (ROS) is a feature integrating normal cellular function as mitochondrial chain respiratory, phagocytosis[3].

The biological systems evolved with the endogenous mechanisms of the defense to help to protect against free radical induced damage of the cell. Glutathione peroxidase, catalase and superoxide dismutase are of the enzymes of the antioxidant that metabolizes intermediates of the toxic oxidative. These enzymes require micronutrient as cofactors as selenium, iron, copper, zinc and manganese for optimum catalytic activity and mechanisms of the defence of the efficient antioxidant mechanisms[2-6].

The trace elements play an important role in the biopsychology of the cells while affecting their growth and contributions to several biologic processes as wound healing. They have been shown to play a preventive measure, diagnosis and curative role like a combatant in several illnesses. They incorporate in the structures of the proteins, the enzymes and the complex carbohydrates to participate in biochemical reaction. Trace elements with enzymes, for example, are necessary for the functioning and maintenance of the immune system[7]. Some trace elements, particularly copper, selenium and zinc, are in fact implied in both humoral and cellular immunity[8]. These essential micronutrients can interfere directly with the propagation stage of free radical generation and scavenge free radicals[5].

Recent studies shows that the products of the plant exert their anticarcinogenic effects by scavenging the free radicals and carcinogen detoxification modulating and detoxification and antioxidant defence system[9]. Indeed, *U. dioica* has becomes a source of folk medicine for treatment of a lot of diseases. The extract of the aqueous methanol of the nettle roots has been used in the clinics for the treatment of prostatic hyperplasia in Europe. The seeds and the aqueous extract of aerial parts of *U. dioica* were used occasionally as medicine makes with herbs by the cancer patients in Turkey[10].

So far, in the literature there is no report related to effect of *U. dioica* on trace elements and serum proteins despite several studies related to the effect of the other parameters on DMBA are available. The aim of the present study was to determine the effect of *U. dioica* on trace elements levels (Cu, Zn, Fe and Mn) and whole serum proteins in white New Zealand female rabbits subjected to DMBA.

EXPERIMENTAL

Animal procedures were approved by the care of the institutional Animals and Use Committee. Adult female rabbits were placed individually in the standard cages in temperature controlled rooms, 12 h light/dark cycles. Food and water were provided *ad libitum*.

The study was performed on 32 white New Zealand adult female rabbits. Average total body weight of subject was between first group 2792-3011 g, second group 2365-2718 g, third group 2721-2959 g, fourth group 2513-2593 g.

Nettle (*U. dioica*) was collected from Van, Turkey and dried at room temperature. Dried aerial material (10 g) was infused in 100 mL of distilled water for 0.5 h. The extract evaporated in vacuum gave a crude residue (yield 21%)[11]. Dried nettle was also extracted with pure methanol[10].

Rabbits were divided into equal four groups. Group I was control group (n = 8), given as i.m. dose of 0.5 mL/kg/d 10 % DMSO in isotonic physiological solution (0.9 % NaCl). Group II (n = 8), treated with 7,12-dimethylbenz(a)anthracene (1 mg/kg.d) in 10 % DMSO as i.m. Group III treated with 7,12-dimethylbenz(a)anthracene (1 mg/kg.d) in 10 % DMSO and methanol extract of *Urtica dioica* 35 mg/kg/d in 2 % Tween-80 solution, and Group IV (n = 8) treated with 7,12-dimethylbenz(a)anthracene (1 mg/kg.d) in 10 % DMSO and aqueous extract of *U. dioica* 35 mg/kg/d in 2 % Tween-80 solution.

Blood samples were collected in 0th, 15th, 30th, 45th, 60th days of period by method of intra-cardiac and centrifuged in 500 g-force for 15 min to obtain sera and later stored at -70 °C until all experimental procedures is carried out.

Serum Zn, Cu, Fe and Mn concentrations were measured using a Solaar atomic absorption (Thermo Electron Corporation, Solaar House, Cambridge England) spectrophotometer. Serum proteins were obtained in groups and were subjected to SDS-PAGE in gel slabs of 1 mm thickness (3.5 cm, 4 % stacking and 15.5 cm, 12 % resolving gels) as described by Laemmli[12]. Electrophoresis was performed with a discontinuous buffer system in a UVP Vertical Electrophoresis Units Cambridge, England. The gel was run at 30 mA until the bromophenol blue marker reached the bottom of the gel. Protein molecular masses were calculated on the basis comparison with the following standards (PageRuler™ Protein Ladder SDS-PAGE Standards, Fermentas, molecular weight range 10-200 kDa). After electrophoresis, the gels rinsed out for 20 min in an isopropanol-acetic acid-water (1:3:6) solution, then for 5 min in methanol-acetic acid-water (3:1:6) solution. Later, the gels were stained for 6 h in 0.01 % (w/v) Coomassie Brillant Blue R-250. Afterwards, the gels were destained in a methanol-acetic acid-water (3:1:6) mixture until protein bands became clearly visible.

Data are presented as means ± SEM (standard error of mean). Differences in biochemical parameters were statistically evaluated using one-way analysis of variance (Anova) followed by Turkey multiple comparison test.

RESULTS AND DISCUSSION

Average serum trace elements of the control, DMBA-treated, aqueous extract of *U. dioica* treated and methanol extract of *U. dioica* treated groups are shown in Table-1.

In statistical analyses, in the 15th day study, levels of group 2 and 4 were compared with the control group levels, a significant decrease was observed in the Zn ($p < 0.05$), ($p < 0.01$), respectively, between levels of group 2 and 4 ($p < 0.01$), in 30th day first and fourth group ($p < 0.05$), in 60th day first and second group ($p < 0.05$).

In the beginning (0th day), levels of group 3 and 4 were compared with the control group levels, a significant increase was observed in the Cu ($p < 0.001$), between levels of group 2 and 4 ($p < 0.01$), 2nd and 3rd group ($p < 0.05$), in 15th day with control 3rd and 4th group ($p < 0.001$), (0.01), respectively, 45th day 1st and 3rd group ($p < 0.05$), in 60th day first group and third group ($p < 0.05$).

Levels of group 4 were compared with the control group levels and between 2 and 4th group in 15th day a significant decrease was observed in the Fe ($p < 0.05$) (Table-1), whereas the Mn value were not found significant ($p > 0.05$) (Table-1).

Fig. 1 shows SDS-polyacrylamide gel patterns of serum proteins in DMBA treated, DMBA-aqueous nettle extract treated, DMBA-methanol nettle extract treated and control groups in periodical times. The analysis of SDS-PAGE indicated that the kind of proteins and their levels in serum not changed among groups in female rabbits.

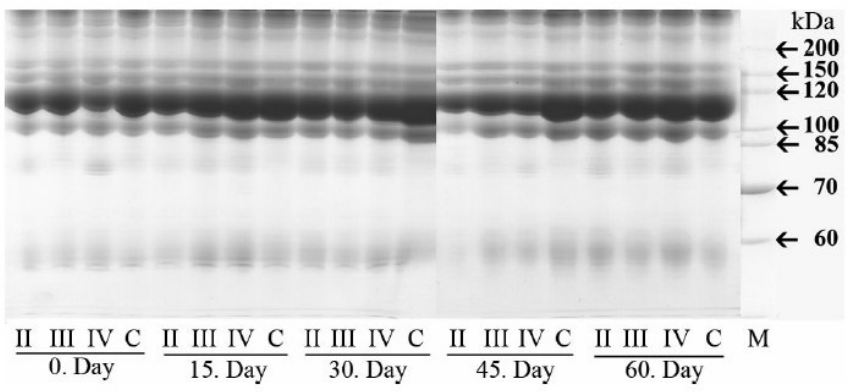

Fig. 1. SDS-polyacrylamide gel patterns of serum proteins, DMBA-treated, DMBA and methanol extract of *Urtica dioica* administrated, DMBA and aqueous extract of Urtica dioica administration groups and control, lines 1-4, day 0; lines 5-8, day 15; lines 9-12, day 30; lines 13-16, day 45; lines 17-20, day 60; lane 14, molecular weight marker ($\times 10^3$ dalton)

TABLE-1
SELECTED TRACE ELEMENTS LEVELS AT DIFFERENT PERIODS IN CONTROL AND ADMINISTRATION WITH DMBA GROUPS IN RABBITS

Groups	0 day, $\overline{X}$ ± SEM	15 day, $\overline{X}$ ± SEM	30 day, $\overline{X}$ ± SEM	45 day, $\overline{X}$ ± SEM	60 day, $\overline{X}$ ± SEM
			Cu (µmol/L)		
Control	6.46 ± 0.88[a,a1]	7.03 ± 0.49[a,b1]	6.51 ± 0.710[c,c1]	6.68 ± 1.380[c]	6.14 ± 0.560[c]
DMBA	9.36 ± 1.95[b,c]	8.83 ± 0.67[b]	9.03 ± 1.310	8.51 ± 1.040	7.66 ± 0.760
DMBA + Nettle (MeOH extract)	18.23 ± 2.45[a1,c]	13.14 ± 0.98[a,b]	12.35 ± 1.419[c]	14.66 ± 2.163[c]	12.51 ± 1.571[c]
DMBA + Nettle (Aqueous extract)	18.31 ± 1.04[a,b]	11.45 ± 0.61[b1]	11.92 ± 1.412[c1]	13.56 ± 1.911	7.91 ± 0.860
			Zn (µmol/L)		
Control	14.91 ± 2.430	16.81 ± 1.09[a,c]	14.99 ± 2.24[c]	14.90 ± 2.00	15.67 ± 2.02[c]
DMBA	14.06 ± 2.406	14.85 ± 1.43[c,b]	13.43 ± 0.81	13.62 ± 0.94	8.06 ± 0.77[c]
DMBA + Nettle (MeOH extract)	10.29 ± 0.600	11.26 ± 0.88	12.05 ± 1.14	13.06 ± 1.03	10.95 ± 1.14
DMBA + Nettle (Aqueous extract)	10.17 ± 0.890	8.97 ± 0.78[a,b]	7.67 ± 1.50[c]	12.87 ± 2.19	12.25 ± 0.59
			Fe (µmol/L)		
Control	29.57 ± 12.53	40.39 ± 7.54[c]	29.63 ± 2.95	37.34 ± 2.55	25.64 ± 5.29
DMBA	25.48 ± 4.73	31.44 ± 4.07[c1]	26.99 ± 3.05	31.55 ± 4.24	23.86 ± 4.45
DMBA + Nettle (MeOH extract)	25.08 ± 4.59	17.05 ± 1.23	21.74 ± 0.99	27.18 ± 3.46	24.28 ± 3.91
DMBA + Nettle (Aqueous extract)	19.23 ± 3.25	13.60 ± 1.40[c,c1]	22.87 ± 4.49	20.91 ± 5.73	18.32 ± 1.29
			Mn (µmol/L)		
Control	1.26 ± 0.32	1.83 ± 0.160	2.11 ± 0.28	1.72 ± 0.29	1.94 ± 0.29
DMBA	0.70 ± 0.17	1.66 ± 0.140	1.75 ± 0.20	1.64 ± 0.31	2.64 ± 0.35
DMBA + Nettle (MeOH extract)	1.17 ± 0.16	1.82 ± 0.330	2.51 ± 0.24	2.11 ± 0.13	2.03 ± 0.21
DMBA + Nettle (Aqueous extract)	1.23 ± 0.22	1.91 ± 0.093	1.99 ± 0.27	1.63 ± 0.20	1.94 ± 0.14

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

The results of the study in 60th day indicated that the serum zinc levels were decreased in treated subjects with DMBA group comparing to control group values ($p < 0.05$), whereas copper levels were increased in DMBA group and methanol extract of *U. dioica* treated group comparing to control values ($p < 0.05$), decreased serum Fe among groups were determined not to be statistically significant ($p > 0.05$). Mn values were not to be changed.

Disilvestro[13] indicated that zinc deficient rats can exhibit high serum levels of acute phase protein ceruloplasmin. The levels raised of this protein can be a sign of inflammation, which is associated with above normal phagocyte secretion rates of free radicals[13]. In another study, Fisher *et al.*[14] have reported that elevation in serum copper appears to be due to elevation in the principal serum cupro-enzyme, ceruloplasmin. It has been suggested that a mechanism of decreased ceruloplasmin catabolism (breakdown) explains the elevated serum copper level in cancer[14]. It is reported that physiologic stress-induced alterations in zinc and copper metabolism have been observed in a lot of studies related to rodents and humans. The acute phase response against stress especially in case of inflammation depresses plasma Zn and raises plasma Cu[15].

Diez *et al.*[16] measured Cu and Zn level in serum patients lung cancer and reported that serum zinc values significantly ($p < 0.001$) lower than those in healthy subjects and serum copper levels higher values than healthy subjects ($p < 0.001$). In this study, elevated copper and decreased zinc level shown consistent with authors[13-15].

Huang *et al.*[17] reported that patients with breast cancer in serum Fe level not significantly higher than healthy subjects. Bhasin *et al.*[18] demonstrate that low iron state reduces the tumor promoting potential of benzoyl peroxide in DMBA initiated murine skin. In this study, decreased Fe values of the group were found not to be in agreement with the values of other researchers[17]. Mn values were not to be changed.

Some researchers[19,20] reported that the level of the expression of several proteins reduced during DMBA-induced HBP carcinogenesis. However, they stated that the level of some plasma proteins did not changed after DMBA-induced HBP carcinogenesis. The present results indicated that nettle extracts did not severally changed in synthesis and levels of plasma proteins all groups in female rabbits subjected to 7,12-dimethylbenz(a)anthracene. These results figure out that nettle did not change the level of some plasma proteins as chemopreventive agent. Besides, it suggested that levels of decreased Zn and Fe and level of increased Cu in rabbits subjected to DMBA might be due to increased free radical generation and acute phase response against oxidative stress. As a results, it was found that nettle extracts not effected on trace element levels and serum protein patterns in rabbits subjected to 7,12-dimethylbenz(a)anthracene.

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