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Effect of Nettle Herb on the Levels of Trace Elements in Rabbits Induced with Dimethylbenzanthracene

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In this study, the effects of nettle herb on the levels of trace elements in rabbits induced with 7,12-dimethylbenzanthracene were studied. Twenty one female New Zealand rabbits were used in the study. The rabbits were divided into three groups. Experiment period was designated as 150 days. The dimethyl sulfoxide (10 %) solution with the dosage of 0.5 mL/kg/day was applied in the control group and 7,12-dimethylbenzanthracene dissolved in dimethyl sulfoxide (10 %) solution was applied as 0.5 mL/kg/day in the 7,12-dimethylbenzanthracene group. In addition to 7,12-dimethylbenzanthracene, the extract of nettle herb was applied as 0.2 mL/kg/day to the other group. The blood samples were taken in the end of the experiment. It was determined that there were varying decreases in all tissues of zinc and manganese levels in the 7,12-dimethylbenzanthracene applied group in comparison with the control data, whereas there were increases in all tissues of iron and copper levels. On the other hand, while zinc concentration decreased in all tissues in the nettle herb + 7,12-dimethylbenzanthracene group, manganese, iron and copper concentrations showed an increase in all tissues.

Key Words: Nettle herb, Trace elements, Rabbit, Dimethylbenzanthracene.

INTRODUCTION

The known name of *Urtica dioica* L. is nettle herb and from Urticaceae family. In recent years, nettle herb has been accepted as a therapeutic plant in Turkey and in many parts of the world because of its effects on human health[1]. In a study conducted by Gülçin *et al.*[2], antioxidant, antiulcer, antimicrobial and analgesic properties of extracts of nettle herb prepared with water were investigated and nettle herb was determined to have a high level of antioxidant efficiency.

Polycyclic aromatic hydrocarbons, known as the most powerful carcinogens, can cause tumors in different tissues and animal species. The most widely used carcinogens in experimental oncology in this group are dibenzanthracene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene[3]. Especially 3-methylcholanthrene (3-MC) and 7,12-dimethylbenz(a)anthracene have been determined to have high levels of immunotoxic effects on *in vivo* animal models and *in vitro* tests conducted with human peripheral blood cells[4].

Trace elements are inorganic substances that participate in catalytic, enzymatic and structural activities in many important events in the organism and that are needed to be taken with food and water from outside. Once entering the organism, trace elements bound to different blood proteins and are distributed to all tissues. Especially Zn, Cu and Mn are important because of their immunity factors in infectious diseases and protective properties against cancer[5]. It is known that variations in levels of the trace element reduce the efficiency of the antioxidant defense mechanism of especially Cu and Fe and increase the negative effects of free oxygen radicals on cell integrity[6].

Copper is essential for many biochemical mechanisms, but it is also a potentially toxic substance[7]. Iron plays a regulatory role in the growth and differentiation of cells[8]. There is a correlation between too much iron uptake and various chronic diseases such as heart diseases[9], diabetes[10] and cancer[11]. It has been reported in the literature that the amount of iron increases in many malignant diseases as well[12,13]. Zinc acts as a neurotransmitter or neuroregulator in the central nervous system[14]. Zinc stimulates gene transcription and cell growth. High Zn concentration may also help the growth of tumor cells[15]. Moreover, Zn is also required for optimal performance of the immune system[16].

The presented study was designed to investigate the effects of nettle herb on trace element concentrations in tissues of rabbits induced with dimethylbenzanthracene.

EXPERIMENTAL

In this study, 21 female New Zealand rabbits were used as animal materials. The rabbits were obtained from Erciyes University, Medical Faculty, Hakan Çetinsaya Experimental and Clinical Research Center. The study was approved by Yüzüncü Yil University, the Ethics Committee of Faculty of Veterinary (Number: 2005/002).

The nettle herb constituting the plant material used in present study was collected in Edremit district, Van province in the months of May-June. Determination of sample species was conducted by Dr. Fevzi Özgökçe (Yüzüncü Yil University, Faculty of Science and Letters, Department of Biology) and the plant samples were recorded in Yüzüncü Yil University, Faculty of Science and Letters, Department of Biology Herbarium with the registration number of S12941.

Preparing the plant extracts: The collected plants were dried in the shade and were finely ground in an electric mill and then were sieved through 0.4 mm mesh. The sieved plant material was kept in colour jars in shade environment until the application. The nettle herb sieved before the application was exposed to extraction in a digestant type Soxhlet device at 50 ºC for 12 h. The extract obtained as a result of the process was evaporated and separated from its solvent methanol. The efficiency of the extract collected in a volumetric flask was found to be 12.5 %. The extract of nettle herb obtained in the study was dissolved in 2 % Tween 80 solution and the nettle herb extract which had 175 mg methanol in 1 mL was applied with the dosage of 0.2 mL/kg/day as IM[17]. After 15 days, the rabbits were taken for the experiment and they were divided into three groups for this purpose.

Preparing the chemicals: 7,12-Dimethylbenzanthracene, used to create toxication in the present study, was dissolved in 10 % dimethyl sulphoxide and this was applied in the form of solution[18].

Experimental procedure: The rabbits were kept for feed and adaptation for 15 days and then they were taken in the experiment. The rabbits were divided into three groups for this purpose.

Control group (n = 7): 10 % DMSO solution prepared with physiological saline water was applied with the dosage of 0.5 mL/kg/day as IM.

7,12-Dimethylbenzanthracene (DMBA) applied group (n = 7): The 7,12-dimethylbenzanthracene dissolved in 10 % DMSO was given with the dosage of 0.5 mL/kg/day as IM.

7,12-Dimethylbenzanthracene + nettle herb extract applied group (n = 7): In addition to 0.5 mL/kg/day 7,12-dimethylbenzanthracene, the nettle herb extract was applied with the dosage of 0.2 mL/kg/day as IM.

The study period was designated as 150 days according to the literature[19].

Statistical method: For the analysis of the obtained data, the Turkey test was used[20].

RESULTS AND DISCUSSION

Zinc, manganese, iron and copper concentrations belonging to the control group and to the 7,12-dimethylbenzanthracene applied group are shown in Table-1, whereas tissue trace mineral levels of the group in which nettle herb + 7,12-dimethylbenzanthracene were applied are shown in Table-2.

Nettle herb acts as an antiinflammatory, antiviral, antioxidant and immune system stimulator and brings about these effects *via* a great number of flavonglycosides and pyrocatechols in its structure. It has been reported that taking pyrocatechols equivalent to the phenols in the nettle herb extract from vegetable and fruit rich diets over daily 1 g can inhibit mutagenesis and carcinogenesis[21].

Polycyclic aromatic hydrocarbons are the known strongest potential carcinogenesis and they can form tumors in various tissues and animal species[22]. They can also form these effects by inhibiting the activities of the immune system[23], suppressing the immune system[24], showing immunotoxic effect[4] and forming free radicals[25].

Zinc, copper and manganese constitute an important part of many enzymes that play an important role in growth, immune system functions, cellular respiration, electron transfer chain and protein synthesis[26]. Copper has important functions in human body. It is the cofactor of superoxide dismutase

TABLE-1
LEVELS OF ZINC, MANGANESE, IRON AND COPPER IN CONTROL GROUP AND 7,12-DIMETHYLBENZANTHRACENE APPLIED GROUP (ppm)

Trace mineral	n	7,12-Dimethylbenzanthracene						
		Control	Kidney	Lung	Muscle	Pancreas	Liver	Heart
Zinc	7	0.616 ± 0.299	0.521 ± 0.145[a]	0.305 ± 0.824[a]	0.276 ± 0.951[b]	0.365 ± 0.132[b]	0.566 ± 0.265[a]	0.281 ± 0.384[a]
Manganese	7	0.0010 ± 0.00	0.009 ± 0.002[b]	0.004 ± 0.001[b]	0.004 ± 0.001[b]	0.005 ± 0.002[b]	0.013 ± 0.005[b]	0.006 ± 0.008[a]
Iron	7	0.018 ± 0.006	0.165 ± 0.074[b]	0.144 ± 0.050[b]	0.195 ± 0.067[b]	0.155 ± 0.142[c]	0.344 ± 0.197[c]	0.131 ± 0.087[c]
Copper	7	0.012 ± 0.001	0.019 ± 0.004[b]	0.014 ± 0.003[b]	0.010 ± 0.002[b]	0.012 ± 0.003[b]	0.016 ± 0.006[b]	0.025 ± 0.007[b]

[a]$p < 0.001$, [b]$p < 0.01$, [c]$p < 0.05$, (in the statistical analyses, experimental groups were compared to the control groups)

TABLE-2
CONCENTRATION OF ZINC, MANGANESE, IRON AND COPPER IN CONTROL GROUP AND NETTLE HERB + 7,12-DIMETHYLBENZANTHRACENE APPLIED GROUP (ppm)

Trace mineral	n	7,12-Dimethylbenzanthracene						
		Control	Kidney	Lung	Muscle	Pancreas	Liver	Heart
Zinc	7	0.6420 ± 0.338	0.456 ± 0.176[b]	0.392 ± 0.257[d]	0.187 ± 0.124[a]	0.383 ± 0.379[a]	0.393 ± 0.714[b]	0.266 ± 0.4530[a]
Manganese	7	0.0005 ± 0.005	0.088 ± 0.017[b]	0.009 ± 0.005[b]	0.153 ± 0.008[b]	0.008 ± 0.001[b]	0.009 ± 0.001[a]	0.005 ± 0.0010[b]
Iron	7	0.0183 ± 0.006	0.124 ± 0.053[b]	0.286 ± 0.284[c]	0.379 ± 0.180[b]	0.211 ± 0.116[c]	0.130 ± 0.033[b]	0.940 ± 0.0269[b]
Copper	7	0.0120 ± 0.001	0.015 ± 0.002[a]	0.012 ± 0.003[b]	0.011 ± 0.001[a]	0.163 ± 0.004[b]	0.015 ± 0.004[b]	0.017 ± 0.0040[b]

[a]$p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$, (in the statistical analyses, experimental groups were compared to the control groups).

(SOD), one of the enzymes protecting the cells from lipid peroxydation and the important element of the mitochondrial cytochrome oxidases. Free Cu acts as a prooxidant agent on cell membranes in the organism[27]. superoxide dismutase protects the cells against free radicals, playing a crucial role in tumor formation. Copper itself, the cofactor of superoxide dismutase, is also a toxic substance and depending on its toxic effect it has been reported to trigger carcinogenesis in the literature[28].

While iron and copper provide the formation of hydroxyl radicals, one of the strong free radicals, *via* the Fenton reaction, they accelerate the transformation of stable lipid hydroperoxides to peroxy and alkoxy radicals. Polyunsaturated fatty acids are present in membrane lipids and they are sensitive to peroxidation[27,29].

In the tissue analyses conducted in the 7,12-dimethylbenzanthracene group and the nettle herb + 7,12-dimethylbenzanthracene applied group in the present study, the zinc levels were observed to decrease in comparison with those of the control groups. On the other hand, it was determined that iron concentrations increased in all tissues in all application groups in comparison with the control data and they were found to be statistically significant in varying ratios.

It has not been clarified exactly whether low zinc levels determined in patients with cancer are the cause or result of this disease. Some researchers suppose that zinc deficiency seen in malignant diseases are the reason of hyperzincuria[30,31] and others think that it is caused by malnutrition[32]. Dobrowolski *et al.*[33] reported increases in the Fe and Cu levels, whereas decreases in the Zn levels in cases of renal cell carcinoma in comparison with normal tissues. Similarly, in studies[34,35] conducted with serums and tissues of the patients with breast cancer and leukemia, while increases have been determined in Cu levels of pathological tissues, decreases have been determined in Zn levels in comparison with normal healthy tissues.

In the tissue analyses conducted in both of the experimental groups in present study, Cu levels were determined to have increases. These increases meant up to $p < 0.01$ statistically significant in the tissues in the 7,12-dimethylbenzanthracene group. On the other hand, the statistical significance was found to be $p < 0.01$ in all tissues except for kidney and muscle ($p < 0.001$) in the 7,12-dimethylbenzanthracene + nettle herb group.

It has been reported in several other studies in the literature that there have been increases in Cu levels in breast cancer, cervical cancers, ovary cancers, prostate cancers, stomach cancer, leukemia and reticulo-endothelial system cancers[36-38].

Manganese is one of the main trace elements and it is one of the structural components of superoxide dismutase. Mn-SOD is an enzyme that should be taken into consideration in terms of its intervention against tumorigenesis and oxidant activities. It has been reported that besides Mn-SOD, there are changes in CuZn-SOD, catalase and glutathione peroxidase in tumor cells[39] as well.

In manganese analyses, decreases were determined in all tissues except for liver tissue in the 7,12-dimethylbenzanthracene applied group in comparison with the control group measurements and these decreases meant up to a statistical significance of $p < 0.01$ in all tissues except for the heart tissue ($p < 0.001$). In manganese analyses conducted in the nettle herb + 7,12-dimethylbenzanthracene study group, increases were determined in all tissue samples in comparison with the control group data and these increases meant a statistical significance of up to $p < 0.001$ in liver and up to $p < 0.01$ in other tissues.

Conclusion

(a) In zinc levels, decreases were determined in all tissue samples in the 7,12-dimethylbenzanthracene group and the nettle herb + 7,12-dimethylbenzanthracene group in comparison with the control data.

(b) In manganese levels, decreases were observed in all tissues except for liver tissue in the 7,12-dimethylbenzanthracene experimental group; on the other hand, increases were observed in all tissue samples in the other group.

(c) In iron concentrations, increases were determined in all tissue samples in both of the experimental groups.

(d) In copper amounts, increases were observed in all tissue samples except for muscle tissue in all experimental groups.

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