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Protective effect of *Hypericum perforatum* L. on serum and hair trace elements in rats 7,12-dimethylbenz[a]anthracene-induced oxidative stress

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ABSTRACT

The study was designed to assess the effect of *Hypericum perforatum* L. (H.P) on serum and hair trace elements and mineral levels, oral administration of 7,12-dimethylbenz[a]anthracene (DMBA) induced oxidative stress in Sprague-Dawley female rats. Analysis of the trace element has been carried out using atomic absorption spectrophotometer method at end of 60th day. It has been found out that the DMBA group contained statistically lower Zn and Cr compared to the control group ($p < 0.01$) and ($p < 0.05$), Cu, Mg and Na contained higher than control group ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$). In DMBA + H.P group, Zn higher and Na lower than DMBA group ($p < 0.05$), ($p < 0.05$), in hair samples Cd, K and Zn contained lower DMBA compared to the control group ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$). In group DMBA + H.P, Cd was higher than DMBA group and Cr lowered accordance with control group ($p < 0.05$). The present study demonstrated significantly positive and beneficial effect of H.P on the concentration levels of Zn and Na in serum, also on Cd levels in hair between DMBA and DMBA + H.P groups.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants found in cigarette smoke condensate, extracts of emissions and indoor air particles, smoky coal combustion, cooking oil fumes, chimney soot, and other

sources (Kleiner et al., 2004). The compound DMBA, a synthetic methylated polycyclic aromatic hydrocarbon, is a potent carcinogen and immunosuppressive agent (Archuleta et al., 1993).

Toxic manifestations of DMBA are associated with its oxidative metabolism that leads to the formation of reactive metabolites (epoxides and quinines) that can generate

Abbreviations: DMBA, 7,12-dimethylbenz[a]anthracene; H.P, *Hypericum perforatum* L.; Cu, copper; Zn, zinc; Fe, iron; Cr, chromium; Mn, manganese; Ni, nickel; Pb, lead; Cd, cadmium; Ca, calcium; K, potassium; Mg, magnesium; Na, sodium; PAH, polycyclic aromatic hydrocarbon.

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free radicals. Various molecules can inhibit the formation of free radicals associated with carcinogenesis. Bioactive compounds from plant origins have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals, they provide protection without causing any side effects and therefore, development of drug from plant products is desired. Many plant extracts and plant products have been identified as good protectors against free radicals by triggering antioxidant gene expression. For that reason natural antioxidants from plant sources have been considered to provide therapeutic drugs (Anbuselvam et al., 2007).

H. perforatum is used in traditional medicine, also has been shown to exert strong antioxidant activity by inhibiting free radical production (Savikin et al., 2007). The major biologically active components of *H. perforatum*, which are considered to be hyperforin (prenylated phloroglucinol) and hypericin (naphthodianthrone), flavonoids and tannins, are also present. *H. perforatum* extract has been shown to protect rats from the inevitable consequences of stress (Barnes et al., 2007). Trace elements play an essential role in many physiological and metabolic processes in the human body as well as in the synthesis and structural stabilization of proteins and nucleic acids. They are also constituents of proteins and hormones. In addition, they are involved in the function of subcellular systems such as mitochondria as well as in membrane transport, nerve conduction, and muscle contraction. Some of them (Zn, Cu, and Mn) act as antioxidants. Therefore, imbalances in the optimum levels of trace elements that may adversely affect biological processes and are associated with many diseases (Rahman et al., 2009).

The aim of this study was to determine the effect of 7,12-dimethylbenz[a]anthracene (DMBA) and *H. perforatum* (*H.P*) on the concentration levels of trace elements (Cu, Zn, Fe, Cr, Mn, Ni, Pb and Cd), and minerals (Ca, K, Mg and Na) in the hair and serum of Sprague-Dawley female rats. The present study also demonstrated that the concentration levels of the minor and the major elements in the hair refracted adequately the mineral status in the serum.

2. Materials and methods

2.1. Extraction of *Hypericum perforatum* L.

H. perforatum was collected from Van, Turkey and authenticated in Department of Biology in Faculty of Science, Yuzuncu Yil University. Plant material was dried at room temperature. Dried material (300 mg) was infused in 30 mL of boiled distilled water for 20 min. After decantation and filtration, the filtrate was again dried in an incubator at a temperature of 50 °C. The aqueous extract was then prepared in isotonic physiological solution (0.9% NaCl) (yield 8.4) (Bnouham et al., 2003; Tahri et al., 2000).

2.2. Animals treatments

Animal procedures were approved by the care of the institutional Animals and Use Committee. Sprague-Dawley female rats were placed individually in the standard cages in rooms

with controlled temperature (22 ± 2 °C), in a relative humidity of $55 \pm 10\%$, and a 12 h light/dark cycles. Food and water were provided ad libitum.

2.3. Experimental design

This study was performed on 24 Sprague-Dawley female rats. Rats were divided into three equal groups. Group 1 was control group ($n=8$), given as orally olive oil. In group 2 ($n=8$), rats were treated with a single dose of DMBA (50 mg/kg) in olive oil given orally. Group 3 ($n=8$), was treated with a single dose of DMBA (50 mg/kg) in olive oil followed by aqueous extract of *H. perforatum* 100 mg/kg day given orally for 60 days.

2.4. Trace element analysis

Hair was taken from each rat and stored at 4 °C until needed for analysis. The hair samples were washed and rinsed four times in a solution containing 1% Triton X-100. The washing procedure was repeated twice and then the hair was dried at 105 °C for 2 h 200 mg of hair samples were measured and kept in a volumetric flask. Digestion procedure was performed pure concentrated acids (nitric acid and perchloric acid 1/5, v/v) 0.2 mL of concentrated trace metal graded nitric acid (65%) 1 mL perchloric acid was added to the hair samples kept in the volumetric flask. The flask was then kept at 80 °C for 7 h. Finally, 1% Triton X-100 and redistilled water was added to make the final volume of 10 mL. The concentrations of elements serum samples were measured in diluted serum. Analysis of the trace element was carried out by using a solar atomic absorption (Thermo Electron Corporation, Solar House, Cambridge, England) spectrophotometer.

2.5. Statistical analysis

The obtained results are presented as the arithmetic mean \pm SEM. The statistical analysis was carried out with analysis of variance (ANOVA) followed by post-hoc comparison of means-Tukey test.

3. Results

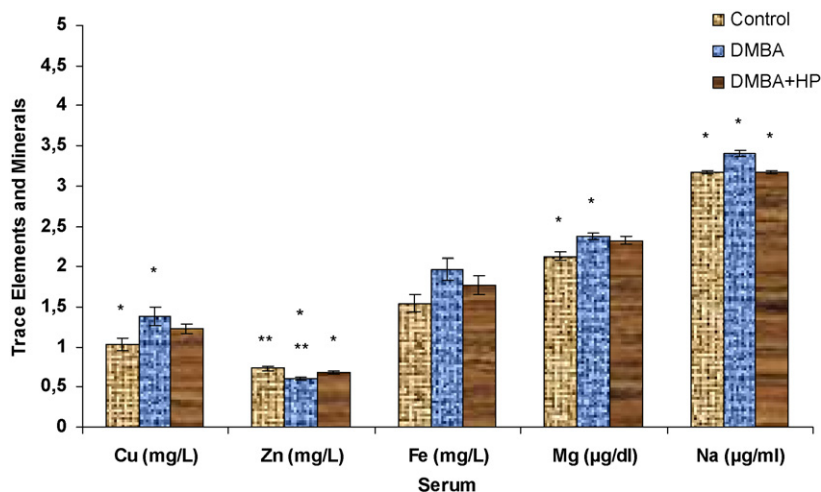
3.1. Serum trace elements and minerals

Table 1 show the results of serum mineral and trace element levels at different periods (0th and 60th day) in control, administration with DMBA and DMBA + *H.P* groups. As it is shown in Table 1, in the beginning (0th day), levels of elements among groups were not found statistically significant. However, in the study of 60th day Zn and Cr levels of DMBA group was compared to the control group levels, a significant decrease has been observed ($p < 0.01$) and ($p < 0.05$), respectively, a significant increase has been observed in Cu, Mg and Na levels ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$), respectively, in DMBA + *H.P* group Zn increased ($p < 0.05$), Na decreased ($p < 0.05$) accordance with the DMBA group (Figs. 1 and 2).

Table 1 – Trace element and mineral levels at different periods in control, administration with DMBA and DMBA + H.P groups in serum samples.

Parameters	0th day ($\bar{X} \pm \text{SEM}$)			60th day ($\bar{X} \pm \text{SEM}$)		
	Control	DMBA	DMBA + H.P	Control	DMBA	DMBA + H.P
Cu (mg/L)	1.11 ± 0.03	1.21 ± 0.14	1.19 ± 0.06	1.03 ± 0.08 ^c	1.38 ± 0.11 ^c	1.22 ± 0.06
Zn (mg/L)	0.95 ± 0.12	1.02 ± 0.10	0.88 ± 0.08	0.73 ± 0.03 ^b	0.60 ± 0.02 ^{b,c}	0.69 ± 0.02 ^c
Fe (mg/L)	1.94 ± 0.15	2.15 ± 0.14	2.12 ± 0.15	1.54 ± 0.11	1.96 ± 0.14	1.77 ± 0.11
Cr (μg/dL)	14.87 ± 1.32	15.26 ± 0.81	14.99 ± 1.64	18.91 ± 1.50 ^c	13.91 ± 1.02 ^c	17.64 ± 0.95
Mn (μg/dL)	2.20 ± 0.51	2.13 ± 1.23	2.59 ± 0.63	3.46 ± 1.49	3.03 ± 0.59	3.61 ± 0.64
Ni (μg/dL)	16.12 ± 1.61	17.73 ± 3.70	14.87 ± 1.88	9.21 ± 2.04	13.56 ± 2.75	9.41 ± 1.57
Pb (μg/dL)	23.15 ± 1.09	20.38 ± 2.88	23.56 ± 4.88	23.40 ± 4.64	31.21 ± 5.37	24.43 ± 2.37
Cd (μg/dL)	3.74 ± 0.18	3.95 ± 0.63	4.18 ± 0.48	6.24 ± 0.34	5.25 ± 0.46	5.24 ± 0.29
Ca (mg/dL)	12.18 ± 0.35	11.75 ± 0.19	12.39 ± 0.22	11.06 ± 0.16	11.50 ± 0.21	11.24 ± 0.17
K (mg/dL)	15.97 ± 0.62	13.86 ± 0.65	14.98 ± 0.73	13.30 ± 0.34	14.49 ± 0.39	14.44 ± 0.79
Mg (mg/dL)	2.54 ± 0.04	2.36 ± 0.08	2.56 ± 0.12	2.13 ± 0.05 ^c	2.38 ± 0.04 ^c	2.32 ± 0.05
Na (mg/mL)	3.42 ± 0.04	3.38 ± 0.04	3.36 ± 0.03	3.17 ± 0.02 ^c	3.40 ± 0.04 ^{c,c1}	3.18 ± 0.02 ^{c1}

^b $p < 0.01$.
^c $p < 0.05$.
^{c1} $p < 0.05$.

**Fig. 1 – Trace element (Cu, Zn and Fe) and mineral (Mg and Na) levels in serum samples of control, DMBA and DMBA + H.P groups in the study 60th day. Error bars indicate the standard error of means. * and ** indicates significance at the $p < 0.05$ and $p < 0.01$ level.**

3.2. Hair trace elements and minerals

Mean values of the trace elements analyzed in hair samples are shown in Table 2. It is seen that in DMBA group Cd, K and Zn levels decreased compared to the control group ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$), respectively, in DMBA + H.P group, Cd increased according to DMBA group and Cr decreased according to control group ($p < 0.05$) (Figs. 3 and 4).

4. Discussion

The aim of this study was to determine beneficial effect of *H. perforatum* on trace element and mineral levels in Sprague-Dawley female rats DMBA-induced oxidative stress. The present study is also presented to evaluate how adequately hair analysis reflects mineral status of experimental animals.

Recently, hair analysis has been used to evaluate the trace element status in the body. Unlike blood, serum and urine, the hair provides historical information on concentrations of trace elements in the body as well as the nutritional condition over a long period of time. In addition, trace elements are often more concentrated in the hair than in body fluids. Furthermore, hair analysis provides information about intracellular accumulation of trace elements. The technique is more invasive for the detection of trace elements, although hair samples can be collected painlessly and stored easily (Ulvi et al., 2002). Using hair as an index of metabolic status of essential trace metals in the body and exposure of the individual heavy metals have attracted considerable interest. In many ways, the hair is ideal for such use, the sample can be readily obtained, without pain or trauma in the patient, is easily collected, and requires no special equipment or storage. The concentration of metals in hair is comparatively high and large samples are normally available, making the task of the analyst easier. In addition,

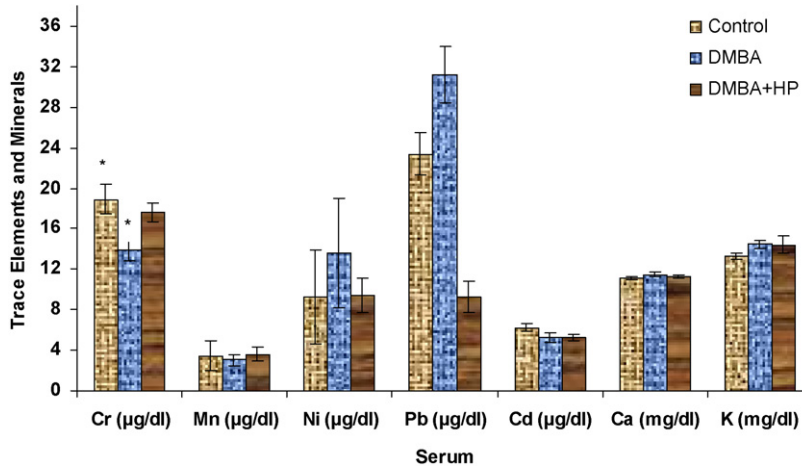


Fig. 2 – Trace element (Cr, Mn, Ni and Cd) and mineral (Ca and K) levels in serum samples of control, DMBA and DMBA + H.P groups in the study 60th day. Error bars indicate the standard error of means. * indicate significance at the $p < 0.05$ level.

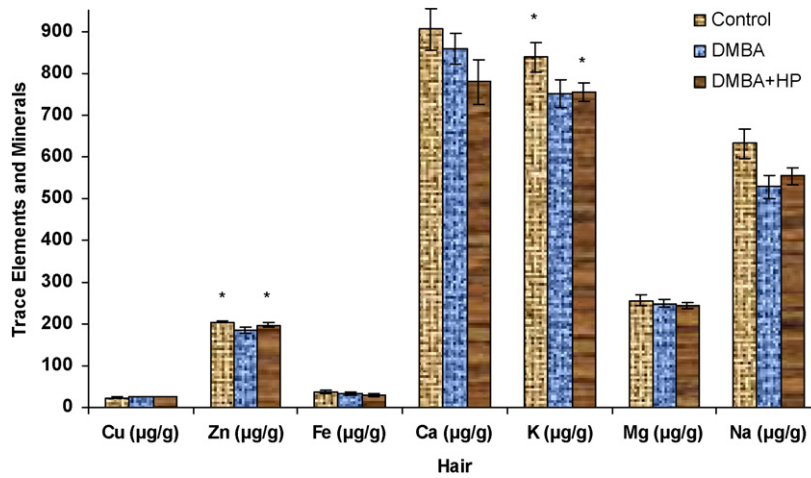


Fig. 3 – Trace element (Cu, Zn and Fe) and mineral (Ca, K, Mg and Na) levels in hair samples of control, DMBA and DMBA + H.P groups in the study 60th day. Error bars indicate the standard error of means. * indicate significance at the $p < 0.05$ level.

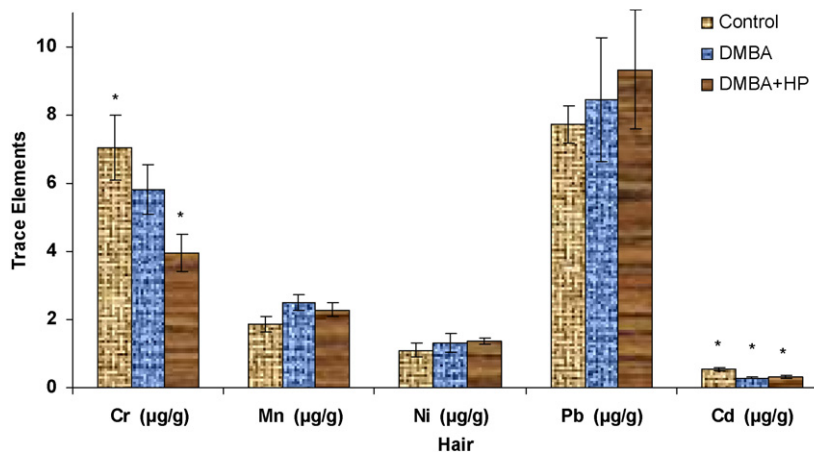


Fig. 4 – Trace element (Cr, Mn, Ni, Pb and Cd) levels in hair samples of control, DMBA and DMBA + H.P groups in the study 60th day. Error bars indicate the standard error of means. * indicate significance at the $p < 0.05$ level.

Table 2 – Trace element and mineral levels in control, administration with DMBA and DMBA + H.P groups in hair samples.

Parameters	60. Day ($\bar{X} \pm \text{SEM}$)		
	Control	DMBA	DMBA + H.P
Cu ($\mu\text{g/g}$)	23.66 \pm 0.59	26.29 \pm 0.55	25.96 \pm 1.35
Zn ($\mu\text{g/g}$)	204.55 \pm 2.29 ^c	184.29 \pm 7.43 ^c	196.45 \pm 5.31
Fe ($\mu\text{g/g}$)	35.60 \pm 3.28	33.99 \pm 3.65	29.13 \pm 2.41
Cr ($\mu\text{g/g}$)	7.06 \pm 0.96 ^c	5.81 \pm 0.73	3.96 \pm 0.55 ^c
Mn ($\mu\text{g/g}$)	1.88 \pm 0.23	2.51 \pm 0.23	2.29 \pm 0.20
Ni ($\mu\text{g/g}$)	1.11 \pm 0.20	1.31 \pm 0.26	1.36 \pm 0.09
Pb ($\mu\text{g/g}$)	7.73 \pm 0.53	8.46 \pm 1.81	9.32 \pm 1.75
Cd ($\mu\text{g/g}$)	0.54 \pm 0.06 ^{c,cl}	0.29 \pm 0.03 ^c	0.32 \pm 0.04 ^{cl}
Ca ($\mu\text{g/g}$)	904.22 \pm 50.35	857.22 \pm 37.28	779.05 \pm 54.41
K ($\mu\text{g/g}$)	838.17 \pm 35.98 ^c	750.18 \pm 31.63 ^c	754.08 \pm 21.49
Mg ($\mu\text{g/g}$)	256.70 \pm 14.29	249.01 \pm 8.23	243.29 \pm 7.92
Na ($\mu\text{g/g}$)	630.80 \pm 36.14	527.92 \pm 27.31	553.10 \pm 21.45

^c $p < 0.05$.
^{cl} $p < 0,05$.

hair does not readily deteriorate and can be stored until it is convenient to perform the analysis. The unique property of hair and nails is that such analyses become especially important when a person may have been exposed to high amounts of a toxic metal (David and Darl, 1974).

It is well-known that the findings of a large number of preclinical researches have documented anti-cancer activities for *H. perforatum* preparations and their constituents. A methanolic extract of *H. perforatum* (containing hypericin 0.3% and hyperforin 3.8%) administered intraperitoneally (15 mg/kg body weight) ten days before the implantation of PC-3 human Caucasian prostate adenocarcinoma cells in nude mice significantly reduced tumor growth and the number of regional lymph node metastases ($p < 0.01$ for both). Moreover, a dried methanol extract of *H. perforatum* protects human neuroblastoma cells against hydrogen peroxide induced apoptosis in vitro (Barnes et al., 2007).

Ciftci indicated that levels of zinc and Magnesium decreased according to control group in DMBA group ($p < 0.01$) and ($p < 0.01$), respectively and increased in DMBA + lipoic acid group ($p < 0.01$) and ($p < 0.01$), respectively in guinea pigs tissue. The level decreased Zn, due to DMBA administration, which may lead to cancer development by increasing oxidative stress (Ciftci and Bakal, 2009).

Wang et al. measured Zn level in serum in patients with breast cancer and reported that serum zinc and Mg values significantly ($p < 0.05$) and ($p < 0.001$), respectively, lower than those in healthy subject (Wang et al., 2006).

The results of study in 60th day serum Zn and Cr levels of DMBA group has been compared with the control group levels, a significant decrease has been observed ($p < 0.01$) and ($p < 0.05$), respectively, Cu, Mg and Na levels was a significant increase has been observed ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$), respectively, in DMBA + H.P group Zn increased ($p < 0.05$), Na decreased ($p < 0.05$) according to DMBA group. In this study decreased zinc and increased Mg levels shown consistent with authors (Ciftci and Bakal, 2009; Wang et al., 2006) due to Zn level, but Mg is not compatible with author (Ciftci and Bakal, 2009; Wang et al., 2006).

On the other hand, it has been observed that mean values of the trace elements analyzed in hair samples in DMBA group Cd, K and Zn levels have decreased compared to the control group ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$), respectively, in DMBA + H.P group, Cd increased accordance with the DMBA group and Cr has decreased accordance with the control group ($p < 0.05$).

Charles et al. has reported that hair K, Ca, Cr, Fe and Zn levels were lower in cervical cancer patients than control group, in contrast Cu level increased in cervical cancer patients compared to control group (Charles et al., 2004). In another study, it has been shown that (Kilic et al., 2004) hair Zn ($p < 0.05$) and Mg levels were lower in breast cancer patients than control, whereas Cu level ($p < 0.05$) was found to be higher in patients with breast cancer compared to control. The present results are consistent with their observations. In another study, Nam-Seok et al. reported that patients with breast cancer in hair Ca, Mg, Fe, Cu, Mn, Zn and Pb levels were lower than in normal controls, but hair Na and K levels were higher in the breast cancer patients than in the normal controls (Nam-Seok et al., 2008). However, in this study it has been shown that decreased hair Ca, Mg, Fe, and Zn levels, in agreement with authors, but Cu, Mn, Pb, Na and K levels have not been compatible with the author.

Experiments on animals have shown that trace elements in diet may influence trace elements in hair. The metabolism of hair follicles which reflects the status of less accessible cells in the body correlations have been found to be between metallic elements in hair and those in certain organs (Klevay et al., 1987).

According to many authors (Unkiewicz-Winiarczyk et al., 2008; Tombarkiewicz, 2008) the most efficient method to estimate the mineral content in the organism is the analysis of element content in the hair.

As a result of this study change in the level of elements has shown that hair is more appropriate than serum analysis in rats which were administered DMBA, and can cause oxidative stress. In conclusion, hair trace elements may often be used for evaluating to the oxidative stress due to alteration among elements and as indices of their body status in experimental animals. The result obtained has shown that the harmful effect of DMBA which is also an environmental factor on animal organisms should be taken into consideration.

5. Conclusions

The present study has shown that extracts of *H. perforatum* had significantly positive effect in serum on Zn and Na ($p < 0.05$) and ($p < 0.05$), respectively, also in hair samples on increased Cd levels between DMBA and DMBA + H.P groups. In conclusion, it suggested that change due to statistical significantly, element level are related with increased free radical generation that is DMBA administration and were seen to have significantly positive and beneficial effects on some elements (Zn and Na in serum, also Cd levels in hair) due to *H. Perforatum*.

Conflict of interest

None.

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