MULTI-ELEMENT DETERMINATION OF MACRO AND TRACE ELEMENTS IN KIDNEY OF DMBA AND LINALOOL APPLIED GUINEA PIGS BY INDUCTIVELY-COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP-AES)

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ABSTRACT

Determination of trace elements in biological samples is important because of their vital role in metabolism, health and oxidative stress. Oxidative stress is caused by reactive oxygen species, depending on many factors in the etiology of various diseases. In our study, protective role of linalool (LIN), which has antioxidant properties in 7,12-dimethylbenz-[a]anthracene (DMBA), administered to guinea pigs was investigated in terms of some macro and trace elements, such as calcium, magnesium, copper, zinc, iron, cadmium and manganese. Determination of these elements in kidney was performed by inductively coupled plasma atomic emission spectrometry (ICP-AES) after microwave-assisted digestion method. Validation of the procedure was examined by using results of recovery experiments. The recovery values of the proposed procedure were found to be between 94.4-101.2%. As a result of the analysis, levels of calcium and cadmium in DMBA group increased when it was compared to control group ($P<0.01$), but was decreased when linalool was used with DMBA. Levels of magnesium, zinc and iron decreased in DMBA group in case of comparison with other groups ($P<0.01$), but increase was obtained in case of using linalool with DMBA. While copper levels between control and DMBA group were not significantly different ($P>0.05$), they increased in DMBA+LIN and LIN groups compared to control group ($P<0.01$). In addition, it was noted that the difference in the levels of manganese between groups was not significant ($P>0.05$). As a result, it can be concluded from the point of macro and trace elements that linalool is effective in the protection of kidney and some other important organs from oxidative stress.

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KEYWORDS: DMBA, 7,12-dimetilbenz-[a]antracene, linalool, trace element, ICP-AES, kidney, animal model.

1. INTRODUCTION

Trace elements play different positive and negative roles for humans, animals and plants [1-3]. Especially zinc, iron, copper, and manganese are known to be essential nutrients for animals, and are needed for nerve impulse transmission, muscle contraction, and cell signaling. Many minerals present in the body, act as helpers in a wide variety of enzymatic reactions [4, 5]. A deficiency in Zn and Cu, especially during pregnancy, may cause organ dysplasia, altered metabolism of proteins and nucleic acids, and impaired fetal development or post-natal growth [6-8]. The symptoms of manganese deficiency include poor growth, skeletal abnormalities, reproductive failure, and ataxia [9]. Cadmium pollution is becoming more common due to its presence in drinking water, cigarette smoke, auto tires and processed meats. It produces pathologies of the lungs, liver kidneys and reproductive organs. When it displaces with zinc, it may cause poor healing and premature aging [3].

Several analytical techniques including neutron activation analysis (NAA) [10], inductively coupled plasma mass spectrometry (ICP-MS) [11], flame atomic absorption spectrometry (F-AAS) [12-15], high performance liquid chromatography (HPLC) [16], and X-ray fluorescence spectrometry [17] have been devised for the determination of trace elements in different matrixes. Methods of NAA and ICP-MS show good sensitivity but usage of these methods are limited because of expensive instrumentation and high cost for routine analysis. AAS and X-ray fluorescence spectroscopy often suffer from the problem of low sensitivity. HPLC has been popularly used for this purpose in recent years, but time of sample preparations is too long. ICP-AES technique performs the simultaneous determination in a large number of elements from

environmental and biological samples, being advantageous from the viewpoint of the short time and the low limit of detection [18].

DMBA is known as oxidant and recognized as polycyclic aromatic hydrocarbon [19]. Due to its carcinogenic effect in relatively high doses, this substance causes tumor development in the body [20], and was reported to significantly increase the free radical level in rat liver [21] and to elevate hydroperoxides in different organs of rats in both short- and long-term administration [22, 23].

Linalool is a monoterpene and reported to be a major component of essential oils in various aromatic species. Several linalool-producing species are used in traditional medicine. Psychopharmacological *in vivo* evaluations of linalool showed to have sedative effects on the central nervous system (including hypnotic; anticonvulsant and hypothermic properties) and many biological activities, such as antioxidant, antimicrobial, anti-inflammatory and anti-tumor properties like vitamin E [24].

It is known that some changes take place in the content of macro and trace elements due to oxidants, such as DMBA, in the organism [3]. Kidneys are mainly affected by acute deposition of the metals. Therefore, we intend to explore the protective role of linalool on trace elements in kidney of guinea pigs exposed to oxidative stress by DMBA. Determinations of elements were carried out by ICP-AES analysis.

2. MATERIALS AND METHODS

2.1. Reagents and solutions

All reagents were of analytical reagent grade (Merck). Ultra-pure water (deionized, 18 MΩ-cm) was obtained using a Milli-Q system (Millipore Corporation, Billerica, MA, USA). All metal solutions were prepared from high purity ICP standard stock solutions (1000 mg L^{-1}) (Merck). Nitric acid (65%) and hydrogen peroxide solutions used were of analytical grade, purchased from Merck.

2.2. Animal protocol and sample preparation

In the present study, 24 healthy, adult, 12-months-old Guinea pigs weighing 550±90 g were obtained from Animal Health and Research Institute (Elazig-Turkey). Animals were reared and maintained according to the standard laboratory conditions (12-h light and 12-h dark cycle; temperature 20±3 ºC). Guinea pigs have been fed with lures, all having the same composition. Four groups (each of 6 animals) were constituted as control, DMBA, LIN, and DMBA+LIN group. DMBA and linalool were dissolved in corn oil. Injections were administered intraperitoneally every day. Only corn oil for control group, 6 mg/kg/day DMBA for DMBA group, 120 mg/kg/day linalool for LIN group, and 6 mg/kg/day DMBA+120 mg/kg/day linalool for DMBA+ LIN group were applied. After a four-week period, the Guinea pigs were decapitated, and their kidneys were re-moved by surgery. Samples of 0.50-0.80 g were taken from the kidneys, washed with isotonic salt solution (0.9 %, v/w) and homogenized. Digestion operations were carried out in the microwave oven in closed system PTFE (polytetrafluorethylene) pots by receiving a 0.30 g homogenized sample according to our previous study [25]. Same operation was applied to the blank solution. Atomic emission spectrometry (Perkin Elmer 3100 ICP-AES) was used in metal determinations.

2.3. Data analysis

Statistical analysis of data was conducted using the variance analysis by ANOVA (SPSS) for comparison between groups. Duncan test was performed to compare the results from different groups. Results are expressed as means ± standard deviation. P-value of less than 0.05 was considered to be significant.

2.4. Analytical features

ICP-AES has been extensively used in the simultaneous determination of major, minor and trace elements in biological material because of its advantages, such as high sensitivity, accuracy, low matrix effect and simpler operation [26].

Calibration curves were obtained from standard stock solutions and metal determinations were performed by using these curves. To evaluate the accuracy of analytical method, recovery experiments were carried out. In these experiments, microwave digestion operation was applied to samples each containing 0.30 g Guinea pig kidney sample and known amounts of standard solutions of metals. After analysis, recovery values were found between 94.4-101.2%. These recovery values have shown that the proposed method gives acceptable results.

3. RESULTS AND DISCUSSION

Trace elements are taken from outside the organism with food, water and some other sources. These elements bind to various blood proteins and are transported to all tissues [27]. The levels of trace elements in Guinea pig kidney are shown in Table 1.

TABLE 1 - The levels of macro and trace element in kidney (µg g^{-1}).

Groups	Ca	Mg	Cu	Zn	Fe	Cd	Mn
control	117.15±24.22^{a}	40.98±8.94^{b}	4.44±0.99^{a}	21.84±3.63^{b}	11.67±1.92^{b}	1.02±0.30^{a}	1.27±0.37^{a}
LIN	129.10±18.73^{a}	32.19±5.71^{a}	5.40±1.70^{b}	20.38±2.82^{b}	13.33±1.98^{c}	1.09±0.35^{a}	1.20±0.36^{a}
DMBA	193.46±24.34^{e}	24.50±5.72^{a}	3.10±1.04^{a}	14.46±1.60^{a}	6.08±0.86^{a}	1.96±0.47^{e}	1.23±0.27^{a}
LIN + DMBA	149.07±18.11^{b}	43.72±7.17^{c}	4.93±1.36^{b}	19.53±1.77^{b}	12.76±2.25^{c}	1.18±0.39^{a}	1.26±0.33^{a}

a,b,c,d: indicate statistical differences in same columns (P<0.01).

It is observed in the present work that Ca levels in the DMBA group are higher than in the other groups (P<0.01). This effect of DMBA was corrected with the effect of linalool in LIN+DMBA group. Statistical difference was not observed in calcium levels between LIN and control groups (P> 0.05). Studies in literature aimed to make relationship between Ca and oxidative stress have shown that level of calcium is higher in damaged tissues and organs relative to normal organs [28].

Free radicals caused by DMBA initiate the lipid peroxidation in the cellular, mitochondrial, nuclear and endoplasmic membranes. It is thought that increase in the permeability causes the calcium leading to mitochondrial damage, to flux and increase its level. It is possible to say that enzymes in defense system become active against the production of free radicals related to calcium by the increase of calcium level in the cell [29].

Magnesium levels in DMBA group decreased according to control group (p<0.01). The level of magnesium in the group of LIN+DMBA has increased relative to DMBA group by the effect of linalool (P<0.01). Thus, it is supposed that negative effects of DMBA on Mg, which has an important role in the stability of cell walls and membranes as well as in the structure of metabolic enzymes [30], can be prevented by linalool.

Although there was no significant difference between the copper levels of control and DMBA groups, it was observed to decrease relatively in DMBA group (P>0.05). The copper levels of LIN and DMBA+LIN groups increased relative to control group (P<0.01). It is detected that the levels of cooper in cancerous and noncancerous kidney tissues are matching (P>0.05) [31].

Level of Zn in DMBA-treated group was significantly lower than that in other groups (P<0.01) and there was not any statistical difference between control, LIN and DMBA+ LIN groups (P>0.05). As Zn has a protective role against formation of free radicals and oxidative stress, its levels were decreased in DMBA group compared to other groups (P<0.01). It was established that linalool corrected this Zn level decrease of the determined amount in combination group whereas it was reported in literature that Zn level in malign endometrium cancerous tissues was lower than that in benign endometrium tissues [32].

The concentration of cadmium in the DMBA group was found to be higher than that in other groups (P<0.01). In literature, it has been pointed out that functions of cadmium are unknown, and it may produce toxic effects even at very low doses. This toxicity could be due to a direct or an indirect interaction which takes place between the metal and the biological system, both extracellularly and intracellularly [30]. Kidney is still regarded as a critical organ for cadmium accumulation and its toxicity. In the kidney, greater than one-third of body Cd deposits were found [33]. It was noted that Zn and Mg had a restoring effect against this damage [27]. In the present study, it is determined that linalool can inhibit the toxic effects of increasing Cd level in organism by increasing the levels of Zn and Mg, and by the effects of other oxidants, firstly DMBA.

It was observed that iron level in DMBA group decreased according to all other groups (P<0.01). Similarly, it was found that the concentration of iron in the tissue of carcinoma kidney is slightly lower than that in normal kidney [31]. Oxidative stress caused by DMBA has decreased the iron holding capability of kidney tissue.

It was stated in a research that increase of accumulation and absorption of cadmium causes the decrease of iron level [34]. The increase of Fe and decrease of Cd thanks to application of linalool emphasize the importance of linalool.

Kidney expresses a range of transport proteins necessary for the reabsorption of filtered essential metals, such as Zn, Ca and Fe. As in the intestine, they are potential routes for Cd uptake. Recent studies indicated that each of these essential metals can inhibit Cd uptake by the proximal tubule [35].

In the present study, it is observed that the concentration of manganese is the same in all groups (P>0.05).

4. CONCLUSIONS

Determinations of Ca, Mg, Cu, Zn, Fe, Cd and Mn are carried out in the kidney of different Guinea pig groups by employing ICP-AES. From the results, it can be concluded that levels of Mg, Zn and Fe are lower (P<0.01) and levels of Mg and Cd are higher (P<0.01) in the kidney of the DMBA-inducted group than control group. These changes of related elements between DMBA and control groups were balanced in DMBA+LIN group with the effect of linalool. Thus, the linalool in oxidant and anti-oxidant studies may play an important protective role.

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