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The Changes in the Serum, Liver, Kidney Protein and Genomic DNA Profiles in Rats Treated with 7,12-Dimethylbenz(A) Anthracene and *Plantago major* L.

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ABSTRACT

In the study, the changes in the serum, liver, kidney protein and genomic DNA profiles in Wistar albino rats administrated to 7,12-dimethylbenz(a)anthracene (DMBA) and *Plantagomajor* L. were investigated. SDS-PAGE profiling of the serum proteins showed that the levels of two proteins molecular weighing 140.8 kDa and 46.8 kDa were significantly lower on the 0th day of treatment than on the 60th day of application in the groups. Furthermore, two proteins (34.5 and 22.3 kDa) were solely present in DMBA-treated liver cell lysates. On the contrary, the kidney protein profiles did not show significant banding variations in groups. Genomic DNA analysis also confirmed that DNA extracted from liver and kidney cells did not fragment in any of the groups after the 60th study day. The proteins 34.5 and 22.3 kDa in liver cell lysates at the 60th day in DMBA-treated group could be extra-prognostic indicators for severe DMBA toxicity.

Keywords: DMBA, *Plantagomajor* L., protein profiles, genomic DNA

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicalsubstances that are generated by incomplete burningof organic materials such as wood, coal, creosote, and crude oil. A mainly origin of PAHs and its derivates isvolcano, forest fires, industrial and domestic waste incineration and various human activities [1]. As a derivative of PAHs, 7,12-dimethylbenz(a)anthracene (DMBA) is hazardously carcinogenic a chemical for mammalian and otherorganisms[2, 3]. DMBA causesnoncancer side effects and several tumors in specific tissues such as kidneys, liver, spleen, mammary and adrenal glands [4]. The available genotoxicity data indicate that there is a correlation between the accumulation of DMBA in different tissues and the development of certain cancer incidences[2, 5].

DMBA and other tumor inducing agents formed many electrophilic reactants which bound to nucleic acids [6, 7], leading to alteration in replication, transcription and causing to tumor formation [8, 9]. DMBA also form adducts with other cellular macromolecules, such as hemoglobin, globin, some large serum proteins [10, 11].Serum include several soluble proteins beneficial for the processes of immunological and cancer studies. Biomarker proteins in serum can be provided useful diagnostic criteria in different tumor-induced carcinogenesis [12-14]. However, it stressed that none of them only was suitable indicator and diagnostic biomarker molecules.

It is known that DMBA induces potential malignant tumors in vitally organs such as liver, kidney, brain and bladder. Especially, the liver is a vital organ of overall metabolism and liver tissue cells metabolize many potentially toxic compounds as *in vivo*. Therefore, the liver is the center of drugs and xenobiotic substances detoxification [8, 15,16].

Medicinal plants have been used by all civilizations from previous time to present because of the healing effects. Ethnopharmacologic studies suggested that natural anti-carcinogenic compounds isolated from plants might be reduced to tumor formation induced by carcinogens [11, 18]. Many studiesconcerningtherapeuticeffects of various medicinal plant extracts have been published related toDMBA-induced carcinoma [19-23]. Furthermore, other study reported that phenolic substances containing in plants can be eliminated to the toxic effects induced by DMBA [24]. *Plantagomajor* (PM) is a perennial vascular plant, growing in the country over the worldwide. PMproduces a number of biologically valuable chemical compounds such as; benzoic acid, ferulic acid, salicilic acid, p-coumaric acid, vanillic acid, baicalein, oleanolic acid and ursolic acid [25]. The aqueous extracts of the PM were used for treating wide range of ailments (diarrhoea, dysentery, cancers, ulcers, bladder problems, gastrointestinal disorders [25, 26]. Additionally, some studies suggested that *Plantago* spp. extracts had inhibitor and cytotoxic effects on melanoma cell lines, breast adenocarcinoma, and Ehrlich ascites tumor cells [18, 27].

So far, we have been conducted a few studies including to levels of total sialic acid, lipid bound sialic acid, some trace elements, retinol, retinylpalmitate, β-carotene, α-tocopherol, vitamin C in rat serum treated with DMBAand *P. major*[28, 29].According to our knowledge in this flied, there has been no data related to effect on serum, liver and kidney protein profiles in the rats administrated with PM and DMBA. Therefore, we investigated the expression levels and variations in liver and kidney tissue protein profiles. The purpose

of the investigation was to determine thedifferentiations in the serum,liver, kidney protein profilesand genomic DNA in Wistar albino rats subjected toDMBA and *P.major* aqueous extract.

MATERIALS AND METHODS

Plant Materials:

Plant samples of *Plantagomajor*were collected from Van, Turkey andidentified and authenticated by the Department of Biology, Facultyof Art and Science, YuzuncuYıl University, Van,Turkey. Plant material dried at room temperature.Dried plant material (300 mg) was infused in 30 mL ofboiled distilled water for 30 min. After decantationand filtration, the filtrate was again dried in an incubatorat 50 ºC. Finally, the aqueous extractwas prepared in isotonic physiological solution(0.9% NaCl) [30].

Animals:

Wistar albino rats were rearedin a room with a constant temperature of 22 ± 1 ºC,a relative humidity of 55 ± 10%, and under the controlled light/dark cycles (12/12 h). Animals were maintained with free accessto water and a standard laboratory diet. The study wasperformed on 18 Wistar albino rats. They were randomly separatedinto three groups of six animals each. Group 1 was used as the control (n = 6) and fed orally with olive oil (intragastrically).Rats in group 2 (n = 6) were treated orallywith a single dose of DMBA (100 mg kg^{-1} bodyweight) in olive oil (intragastrically). Animals in Group 3 (n = 6) was treated orally with a single dose of DMBA(100 mg kg^{-1} body weight) in olive oil followed byaqueous extract of PM (100 mg kg^{-1} body weight)every day (intragastrically). The administrationswere performed throughout 60 days for each group. All procedures were carried out in accordance with the conventional guidelines of the Local Institutional Committee, Van, Turkey, for experimentation with animals.

Preparation of Serum and TissueSamples:

Blood samples were collected at the beginning ofapplication (0^{th} day) and on the 60^{th}study day of administrationby theintracardiac method and centrifuged at 500 rpm for15 min to obtain the sera.Later, each the serum sample (20 µL) was transferred into an eppendorf tube,and adding 25 µL of SDS-sample buffer containing 0.006 M Tris-HCl, 2.5% glycerol, 0.5% SDS, and 1.25% β-mercaptoethanol (pH 6.8), and the mixture was stirred gently to ensure good homogenization. Then the serum proteins were denatured in a boiling water bath for 5 min., and the denatured proteins were stored at –70 ºC until the electrophoresis was carried out. Liver and kidney samples following 60^{th} study day were just excised and washed in ice-cold 1.15% KCl solution, dried using filter paper. Later, they homogenized in 5 folds of 50 mM Tris-HCl buffer (pH 7.5).After centrifugation at 12.100 rpm for 10 min.,25 µL of the supernatant was transferred into an eppendorff tube. Finally, 25 µL of denaturing buffer (0.006 M Tris-HCl, 2.5% glycerol, 0.5% SDS, and 1.25% β-mercaptoethanol, pH 6.8)added to the supernatant samples, and proteins were denatured in boiling water for 5 min. The denatured tissue proteins were keep at –70 ºC until the electrophoresis was carried out.

SDS-PAGE of Serum and TissueProteins:

The serum and tissue proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 1 mm thick gel slabs (3.5 cm, 4% stacking and 15.5 cm, 12% resolving gels) as mentioned by Laemmli [31]. Electrophoresis was performed with a discontinuous buffer system in a UVP vertical electrophoresis unit (Cambridge, England). The gel was run at 30 mA until the bromophenol blue marker reached the bottom of the gel. Protein molecular weights were calculated by comparison with the following standards (PageRulerTM Protein Ladder SDS-PAGE Standards, Fermantas, molecular weights range 10-200 kDa). After electrophoresis, the gels were rinsed for 20 min. in 1:3:6 isopropanol-acetic acid-water solution and then for 5 min in 3:1:6 methanol-acetic acid-water solution. Finally, the gels were stained for 6 h in 0.01% (w/v) Coomassiebrillant blue R-250 followed by destaining in the 3:1:6 methanol-acetic acid-water mixture until the protein bands became clearly visible.

The gels were scanned via high resolution scanner (HP 3500 C Hewlett Packard Co.), and the molecular weight of each protein band was determined by one-dimensional analysis software (Total Lab 1D Manual R11.1, UK).

DNA Extraction and Agarose Gel Electrophoresis:

DNA extraction from liver and kidney tissues were carried out according to Sambrook et al. [32].Extracted DNA was dissolved in loading buffer (10 mMTris-HCl, pH 8.0, 1mM EDTA, 50µg/mLRNase). DNA for each sample (5 µg) was loaded into1% agarose gel in 89 mM Tris-borate, 89 mM boricacid and 2 mM EDTA pH 8.0).Genomic DNA subjected to electrophoresis at 100 V 2 h on a horizontal slab gel unit (Midicell® Primo Submarine Gel System, USA) and stained with ethidium bromide for photography under UV illuminator (Cedex, France).

RESULTS

SDS-PAGE profiles of serum proteins of the control group, DMBA-treated group and PM aqueous extract-treated group at the beginning of application and on the 60th study day are shown in Figure 1. The levels of two proteins molecular weighing 140.8 kDa and 46.8 kDa (marked as 2 and 6) were significantly higher on the 60thday of treatment than on the 0thday of application for whole groups.However, the levels of fourproteins numbered by 1,3,4 and 5, respectively were not shown significantly a changebothon the 0th and 60th study days following the administrationfor each group.

Figure 2 are shown the SDS-polyacrylamide gel protein profiles of liver and kidney tissues inthe control group, DMBA-treated group and PM aqueous extract-treated group after the 60th study day. The results of SDS-PAGE analysis indicated that the high (186.2 kDa, numbered by 7) and the low (34.5 and 22.3 kDa, marked as 8 and 9) molecular weight polypeptides of liver were appeared only in DMBA-treated and PM+DMBA-treated groups.On the contrary, the levels of kidney protein profiles were not seen significantly banding variationsforall groups.

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Additionally, the results of agarose gel electrophoresis analysis showed that the genomic DNA of liver and kidney tissues not fragmented in the control group, DMBA-treated group and PM aqueous extract-treated group after the 60th study day (Figure 3).

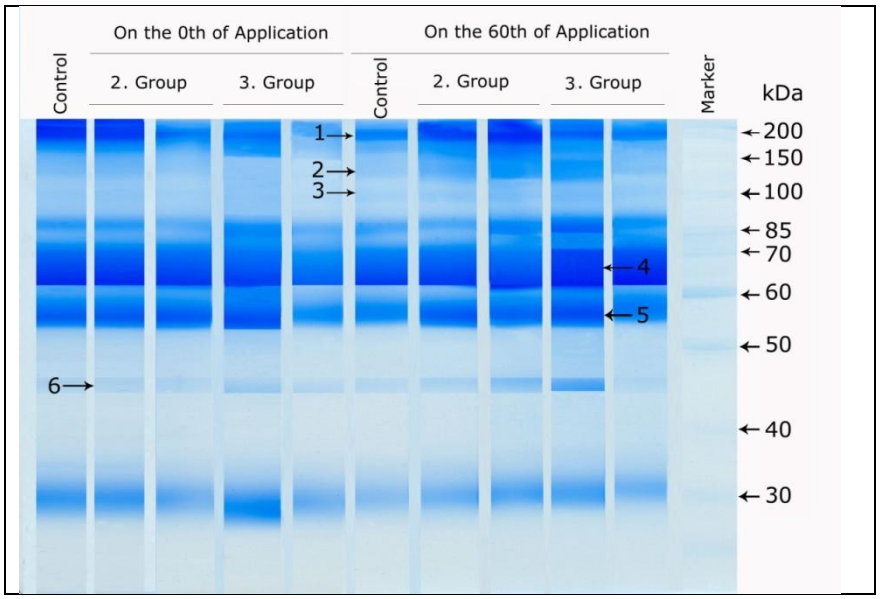

Fig. 1. SDS-PAGE profiles of serum proteins of 1. Group (Control), 2. Group (DMBA) and 3. Group (DMBA+PM). Lines 1-5, 0th day; lines 6-10, 60th day, line 11, marker.

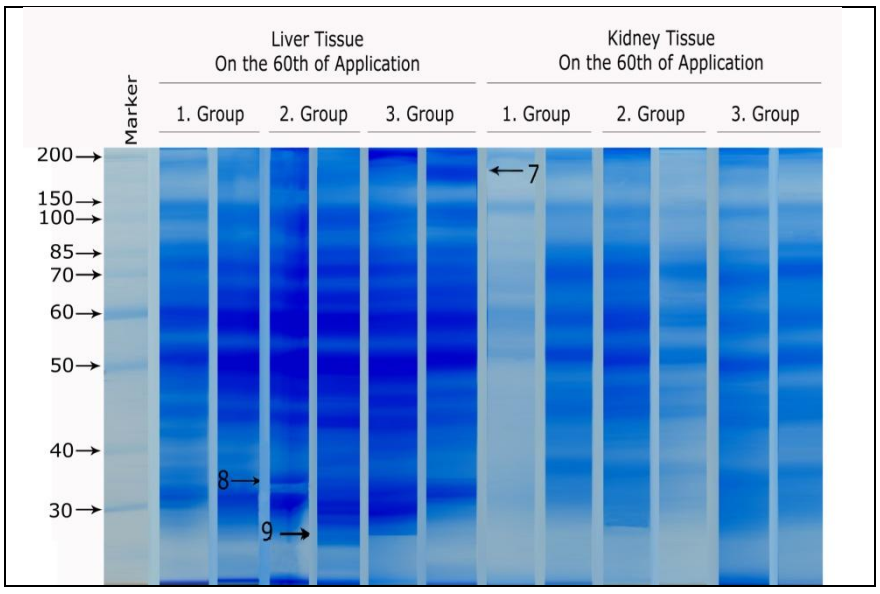

Fig. 2. SDS-PAGE profiles of liver and kidney tissue proteins of 1. Group (Control), 2. Group (DMBA) and 3. Group (DMBA+PM). Line 1, marker; lines 2-7 0th day; lines 8-13, 60th day.

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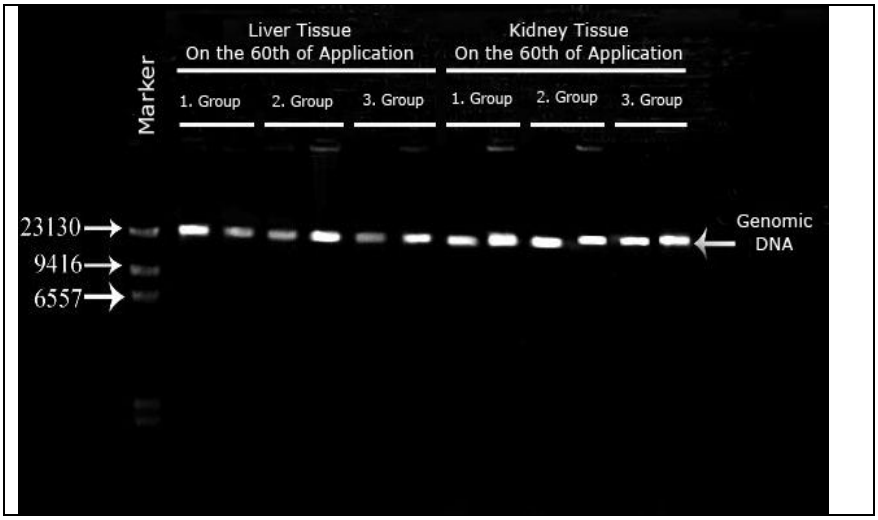

Fig. 3. Genomic DNA profiles of 1. Group (Control), 2. Group (DMBA) and 3. Group (DMBA+PM). Lines 1, marker; lines 2-7 0^{th} day; lines 8-13, 60^{th} day.

DISCUSSION

As a derivative of polycyclic aromatic hydrocarbons (PAHs), 7,12-dimethylbenz(a)anthracene (DMBA) is one of the most important harmful and carcinogenic substancefor humankind [2, 3]. The compound causedtumors in some specific tissues such as kidneys, liver, spleen, mammary and adrenal glands because of stored mostly in the sites[4].In the present study, we investigated the changesin serum, liver and kidney tissue protein profiles and genomic DNA in Wistar albino rats administrated to DMBA and *P. major*.

Serum contain a number of soluble proteins useful for the processes ofimmunological and cancer investigations.Biomarker proteins in serum can be provided usefuldiagnostic criteria in different tumor-induced carcinogenesis[12-14]. However, it stressed that none of them solely was suitable indicator and diagnosticbiomarker molecules.In this study, SDS-PAGE profiling of the serum proteinsshowed that the levels of two proteins molecular weighing 140.8 kDa and 46.8 kDa were significantly lower on the 0^{th}day of treatment than on the 60^{th}day of application in all groups. On the other hand, the levels of four proteins molecular mass between 50-200 kDa did not changeconsiderably in each group throughout all the administrations. Our finding confirmed that the expression levels of a few serum proteinschanged in DMBA-induced toxicity in rats.However, it was also observedthat the administration of *P.major* aqueous extract didnot crucially affectedin serum protein profiles of animals in Group 3 on the 0^{th} and 60^{th} study days.Our findings are good agreement with previous studies[12, 14, 19, 21].

In recently years, a number of valuable papers including protective effects of aqueous extracts of different medicinal plants have been published about DMBA-induced carcinoma[4, 19-23]. The results of these studiesrevealed that there was a decrease in the level of the expression of some specific proteins and an increase expression of some proteinsin various carcinogenesis induced by DMBA.Additionally, some studies suggested that *Plantago* spp.extracts had inhibitor and cytotoxic effects on melanoma cell lines, breast adenocarcinoma, and Ehrlich ascites tumor cells [18, 27]. It is shown known that DMBA

induces potential malignant tumors in some vitally important special tissues such as skin, kidney, mammary, bladder, brain and liver. Therefore, we also investigated the expression levels and variationsin liver and kidney tissue protein profiles.

The liver isone of the important organ of overall metabolism and liver tissue cells metabolize various potentially toxic compounds. Therefore, the liver is the center of drugs and xenobiotic substances detoxification[8]. Alterations in hepatic metabolism may affect the balance between the levels of detoxification products and carcinogenic metabolites[15, 16].In the present study, two proteins (34.5 and 22.3 kDa) determined in DMBA-treated liver homogenates may be extra-prognostic indicator for DMBA toxicity. It was also evaluated that the presence of the protein molecular weighing 186.2 kDa in DMBA+PM group was incoherent.On the other hand, in the kidney protein profiles were not seen significantly banding variations among groups, in spite of the changes in the expression levels of some proteins.

The data gathered from many studiessuggested that DMBA and similar tumor promoting agents formed various electrophilic reactants which bind tightly to nucleic acids [6, 7], leading to alteration in replication, transcription and causing to tumor formation[8, 9].In this study, we have not observed a significant change in the nuclear DNA isolated from liver and kidney cells for all three groups following the 60^{th} study day.Our findings are agreed with partially Oommen et al. [33] and previous studies[8, 15].

In conclusion, our results suggested that the increases in the amount of the proteins weighing 140.8 kDa and 46.8 kDa in serum at the 60^{th} day in DMBA-treated and DMBA+PM treated groups might be linked in developing DMBA toxicity. Additionally, the proteins weighing 34.5 and 22.3 kDa at the 60^{th} day in DMBA-treated for liver homogenates could be extra-prognostic indicator for severe DMBA toxicity. However, we suggested that to obtainingmoreaccurate prognostic tools related to the DMBA toxicity will be necessary more comprehensivedata.

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