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Immunohistochemical evidence of lipid peroxidation role in diethylnitrosamine-induced hepatocellular carcinoma in male Wistar albino rats

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Abstract

This study aimed to evaluate malondialdehyde (MDA) expressions using the immunohistochemical method in order to reveal the role of lipid peroxidation in the development and progression of hepatocellular carcinoma (HCC) induced by diethylnitrosamine (DEN) in male Wistar albino rats. Avidin-biotin-peroxidase method was used for immunohistochemical evaluation. Histopathological examinations revealed that DEN caused a mixed pattern (trabecular and acinar formations) of HCC in the majority of rats. The MDA positive stainings were significantly increased in rats in the HCC group compared to the healthy rats in the control group. In conclusion, this study data contain three important findings. The first one is that DEN triggers the formation of reactive oxygen species (ROS), excessively produced ROS cause oxidative stress, and as a result, oxidative stress strongly causes lipid peroxidation. Secondly, it is clear that there is an important relationship between oxidative stress-induced lipid peroxidation and HCC progression. At the same time, MDA is an useful biomarker in determining the prognosis of patients with HCC. The third and final finding is that intra-peritoneal DEN injection once a week for 20 weeks, but not in combination with other promoting chemical agents, appears to be very effective in inducing experimental HCC.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver (75.00 to 90.00% of cases). The other type of cancer seen after HCC is intra-hepatic cholangiocarcinoma (10.00 to 15.00%).[1-3] The HCC is the fifth most common cancer in the world and accounts for approximately 5.00% of malignant tumours in humans.[4] While the incidence of HCC is the highest in Asia, the incidence of cancer is reported to be quite low in Europe.[5] The HCC has a high mortality,[6] and is typically a result of cirrhosis, leading to cancer-related deaths, ranking as the second leading cause of such deaths.[7] Additionally, the incidence of HCC is expected to increase in the coming years.[8]

Diethylnitrosamine (DEN) is one of the most important environmental carcinogens.[9] It is frequently found in salted fish, meats, alcoholic beverages, cosmetic products, pesticides, and cigarette smoke.[10,11] The DEN can cause cancer in many organs, such as skin, stomach, and lungs,[12] and is mostly used to trigger HCC in experimental animals.[8] The HCC generated as a result of DEN in animals is both histologically and biochemically similar to the HCC in humans.[13] The DEN not only induces HCC but can also promote it.[14] It exerts its carcinogenic effect through two different mechanisms. The first of these is DNA alkylation, causing DNA damage and subsequent cell degeneration.[3,5] The second is the activation of hepatocyte cytochrome p450, inducing the reactive oxygen species (ROS) formation.[15] The DEN causes oxidative stress and cellular damage by increasing the formation of free radicals.[16] The carcinogenic effect of DEN is related to the ROS produced at high rates as a result of metabolic biotransformation.[17] The ROS interact with biomolecules, such as DNA, proteins and lipids, and cause serious damage to them.[1]

Oxidative stress represents the imbalance between ROS production and anti-oxidant defence systems.[18] Biomolecules and macromolecules in cells are the main targets of oxidative stress, leading to lipid peroxidation by membrane disruption, nuclear breakage, chromosomal abnormalities, and changes in intra-cellular signalling pathways and gene expressions.[19,20] These modifications in oxidative stress play a critical role in hepatocarcinogenesis.[21] Oxidative stress leads to peroxidation of

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membrane lipids, and this process produces various lipid oxidation products, such as malondialdehyde (MDA), a low-molecular-weight aldehyde produced by the attack of free radicals on polyunsaturated fatty acids during cellular membrane phospholipid degradation.[22,23] The MDA is a highly potent carcinogen and reacts with DNA to produce MDA-DNA adducts, being involved in the induction of G→T transversions and A→G transitions.[20,24] The MDA is also an important biomarker widely used to determine lipid peroxidation.[22]

This study aimed to evaluate MDA expressions using the immunohistochemical method in order to reveal the role of lipid peroxidation in the development and progression of HCC caused by DEN in male Wistar albino rats. Literature reviews show that many promoter chemical agents are applied synchronously after DEN injection in experimental HCC studies. However, the current study will reveal the effectiveness of DEN injection alone in inducing HCC without the need for other promoter chemical agents. In this respect, it is thought that the data obtained from this study will contribute to the literature, specifically in experimental liver cancer model studies.

Materials and Methods

Ethics committee certificate, animals and experiment setup. The study was approved by the Local Ethics Committee of Animal Experiments of Kafkas University, Kars, Türkiye (Approval No. KAU-HADYEK: 2021-043, Date: 25.03.2021). The study material consisted of twenty 2-month-old male Wistar albino rats weighing an average of 350 g obtained from the Experimental Animal Unit of the Veterinary Control Institute of Erzurum. The experimental animals were divided into two different groups, with 10 rats in each group. The rats were housed in the Laboratory of the Experimental Animal Application and Research of Kafkas University under appropriate conditions, such as 23.00 ± 2.00 ˚C, 55.00% humidity, and a 12-hr day and night cycle. Standard feed and drinking water were given to the rats *ad libitum* during the study. The experimental groups in the study were as follows: Control group: Rats were given pellet feed and water for 20 weeks and HCC group: *N*-nitrosodiethylamine (Sigma Aldrich, St. Louis, USA) was administered intraperitoneally once a week for 20 weeks at a dose of 50.00 mg kg^{-1}. At the end of the study, the experimental animals were euthanized under general anesthesia using 80.00 mg kg^{-1} pentobarbital (Bioveta, Ankara, Türkiye) intraperitoneally by cervical dislocation. Then, systematic necropsy of the animals was performed and liver tissues were taken.

Histopathological examinations. Liver samples obtained from the rats were fixed in a 10.00% formaldehyde solution. Serial sections with a thickness of 5.00 µm were taken from the paraffin blocks prepared after routine tissue follow-up procedures. The sections were stained with Hematoxylin and Eosin to detect histopathological changes in the tissues. The prepared sections were analyzed in detail under a light microscope by at least two different pathologists, and photographs of the recorded histopathological changes were taken for each case.[25]

Immunohistochemical examinations. Serial sections with a thickness of 4.00 µm were taken from paraffin blocks prepared from liver tissues and stained using Avidin-Biotin Peroxidase echnique and MDA commercial antibody (pre-treatment: Microvawe oven, Abcam, Cambridge, UK, polyclonal, 1/1,500, overnight, and 4.00 ˚C) according to the manufacturer's procedure. Histostain IHC Kit (Thermo Fisher Scientific, Waltham, USA) was used for all the immunohistochemical stainings. The 3.3-diaminobenzidine tetrahydrochloride solution (Thermo Fisher Scientific) was chosen as a chromogenic substrate, and it was dropped onto the sections and incubated for 15 min. The sections were washed with distilled water for 5 min, stained with Mayer Hematoxylin, and covered with immune mount.[26] After the covering process, the preparations were evaluated under a light microscope (Olympus, Tokyo, Japan), and photographs of the sections were taken with the Cell ^P program (version 3.4; Olympus, Münster, Germany). The photographs taken were analyzed using the ImageJ Software (National Institutes of Health, Bethesda, USA).

Immunohistochemical staining analysis Immune positive reactions were analyzed, basically, with a grading system created by considering the number of positive cells in the foci examined exhibiting the strongest staining character. Analysis of the quantification of immune-positive reactions in liver tissues was started based on strongly stained areas. For each experimental animal, three different fields were examined under a 50 × lens. The number of cells showing positive reactions was recorded separately, and the average of these three distinct areas was determined as the average positive cell number of that experimental animal. Rating was defined as (-): No immunoreactivity, (+): Weak, 1.00 - 10.00% positivity, (++): Moderate, 11.00 - 59.00% positivity, and (+++): Severe, over 60.00% positivity.[27]

Statistical analysis. The data obtained were analyzed using the SPSS Software(version 20.0; IBM Corp., Armonk, USA). After the Shapiro Wilk normality test, differences between groups showing non-parametric distribution were determined by the Mann-Whitney U test ($p < 0.05$).

Results

Histopathological findings. The histopathological evaluations of rats belonging to the control and HCC groups are summarized in Table 1. It was determined that the livers of the rats in the control group exhibited a

normal histological structure, and no notable pathological conditions were observed in these animals (Fig. 1A). A strong HCC formation was observed in the liver in the DEN-applied group. In this group of animals, many cancer areas of varying sizes were observed in the liver tissue, surrounded by a fibrous capsule, with borders clearly separated from the normal tissue. It was observed that neoplastic cells spread to different points of these areas and formed acinar and trabecular formations. While, in some cases, the tumoral areas consisted mostly of acinar structures, in other cases it was determined that trabecular structures were much more dominant in the area. However, when the general picture was observed, it was noted that the mixed pattern in which trabecular and acinar structures coexist was much more dominant in HCC cases. There was a significant pleomorphism in the tumoral foci. It was determined that the nucleus/ cytoplasm ratio of cancer cells increased in favour of the nucleus. However, the nuclei of the neoplastic cells were markedly hyperchromatic. It was also observed that the nuclei of the tumoral cells were eosinophilic. In some cells, the increase in the number of nucleoli was remarkable. Moreover, it was noted that mitotic figures increased dramatically. There were large areas of necrosis and bleeding in the middle of the tumoral foci. In addition, bile duct proliferation and connective tissue increases were other histopathological findings observed (Fig. 1B).

Immunohistochemical findings. No reaction was observed regarding MDA staining in the livers of the rats in the control group (Fig. 2A). The MDA-positive staining was significantly increased in the rats in the HCC group compared to the control group ($p < 0.05$). The MDA-positive staining was much stronger in the tumoral areas that had reached larger sizes compared to the small nodules surrounded by a fibrous capsule. The MDA expressions were mostly concentrated at the periphery of tumoral foci. However, MDA-positive staining was detected in the cytoplasm of cancer cells forming trabecular and acinar formations. Granular intra-cytoplasmic staining was dark brown. The intensity of staining was much more dominant in macro-trabeculae than micro-trabecular structures. It was noted that the number of MDA-positive cells was much higher in the mixed pattern in which trabecular and acinar structures were present together, compared to the cases exhibiting only a trabecular or acinar pattern (Fig. 2B).

Table 1. Histopathological examinations and malondialdehyde immunoreactivities of the cases belonging to the experimental groups.

Groups	Number	Pattern	Malondialdehyde
Control	1	Normal	0
	2	Normal	0
	3	Normal	0
	4	Normal	0
	5	Normal	0
	6	Normal	0
	7	Normal	0
	8	Normal	0
	9	Normal	0
	10	Normal	0
Hepatocellular carcinoma	1	Trabecular	2
	2	Acinar	2
	3	Trabecular + Acinar	3
	4	Acinar	2
	5	Trabecular + Acinar	3
	6	Trabecular + Acinar	3
	7	Trabecular + Acinar	3
	8	Trabecular	1
	9	Trabecular + Acinar	3
	10	Trabecular + Acinar	2

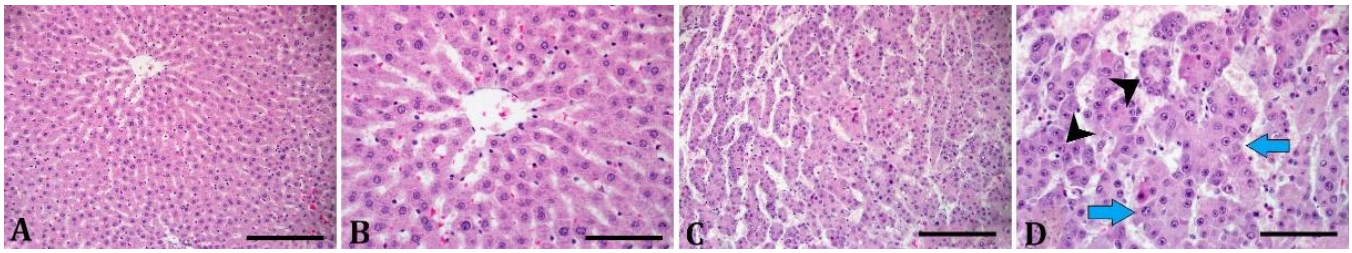

Fig. 1. Photomicrographs of liver tissue in experimental groups. **A** and **B)** Control group; **C** and **D)** Hepatocellular carcinoma group, acinar formations (black arrowheads) and trabeculae (blue arrows) can be seen (Hematoxylin and Eosin staining, bars = 100 µm in A and C, 50.00 µm in B and D).

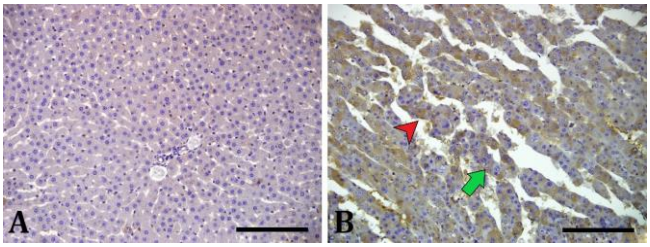

Fig. 2. Immunohistochemical examination of liver tissue in experimental groups. **A)** Control group, negative malondialdehyde (MDA) expression; **B)** Hepatocellular carcinoma group, intra-cytoplasmic MDA positive stainings in acinar structures (red arrowhead) and trabeculae (green arrow) are evident (bars = 100 µm).

Discussion

Metabolic activation of DEN by cytochrome p450 enzymes converts it to ethyl-acetoxyethyl-nitrosamine, which can be conjugated by phase II enzymes to a non-toxic compound. Alternatively, it can form ethyldiazonium ion, which directly ethylates cellular macromolecules and is responsible for their cytotoxic, mutagenic, and carcinogenic effects through ROS production.[16] The ROS formed during the metabolic biotransformation of DEN suppress the anti-oxidant defence system and cause oxidative stress.[20,28] Oxidative stress causes genomic damage and genetic imbalance, causing mutations.[21] These mutations caused by oxidative stress play a role as a triggering factor in both the initiation and progression of carcinogenesis.[19] The DEN-induced HCC model is very similar to HCC in humans.[6] The liver cancer model created as a result of DEN is accepted and widely used worldwide.[1,28] The hepatotoxic and carcinogenic effects of DEN in rats are applied systematically at the weaning stage through water drinking, oral gavage, and intra-peritoneal applications.[12] Although it is recommended to be administered together with promoter agents, such as carbon tetrachloride, this study revealed that intra-peritoneal DEN administration alone for 20 weeks successfully and effectively caused HCC in all 10 rats.[3,17] Hussein *et al.*[8] have observed that moderately differentiated HCC accompanied by thick trabecular structures forms in the liver as a result of 14 weeks of intra-peritoneal DEN injection. In another study, Vanli *et al.*[29] have found that both trabecular and pseudoglandular structures are present together in cancer foci in an experimental HCC model study in which DEN and other chemicals were applied together. Similar to this research, it was determined by histopathological evaluations that a mixed pattern of HCC was mostly formed in rats, mostly with micro- and macro-trabeculae and acinar structures.

It is known that high ROS production causes DNA damage, mutations in tumour suppressor genes, gene disorders, and damage to the fatty acid side chains of lipids located in cell membranes.[18] Lipid peroxidation is a process initiated by the free radical attack on one or more fatty acids, producing a wide range of toxic and reactive metabolites, such as MDA and 4-hydroxynonenal.[7] These metabolites directly attack DNA and cause mutation and carcinogenicity.[14] Lipid peroxidation, one of the most important cellular consequences caused by uncontrolled ROS increase, can be detected mostly through MDA levels. The MDA, which has highly mutagenic and carcinogenic effects, reacts directly with DNA to form oxidative DNA adducts, thus causing DNA damage and affecting the DNA repair mechanisms.[2,10,16] There are several studies investigating lipid peroxidation using various methods in DEN-induced liver cancer in rats. Ramakrishnan *et al.*[24] have demonstrated by immunohistochemical methods that in the rat model of DEN-induced hepatocarcinogenesis, MDA expressions increase significantly compared to the control group. Singh *et al.*[20] have noted that hepatic lipid peroxidation values in the liver of rats in the DEN-induced cancer group are statistically higher than those of the control group. In a similar study, Jayakumar *et al.*[28] have reported that lipid peroxide levels increase dramatically in rats with DEN-induced HCC. In another study, Fahim *et al.*[13] have observed that DEN application increases MDA levels compared to the healthy control group. Similarly, Hussein *et al.*[8] have pointed out the significant increase in MDA levels in rats with HCC, confirming the current study data. In the current study, similar to the reports of other researchers,[8,13,20,24,27] it was revealed through MDA expression that DEN administration led to a significant increase in lipid peroxidation compared to the control group. It is clear that such an increase in MDA levels is linked to the high levels of ROS that occur during DEN metabolism. In human medicine, there are many studies reporting MDA levels in patients with HCC.[23] Lorente *et al.*[22] have reported that MDA levels indicate poor prognosis for HCC patients. Elbaz *et al.*[23] have also reported that serum MDA levels increase in HCC patients compared to the cirrhotic patients, and they have also noted that there is a positive correlation between tumour size and MDA levels. It has been reported that blood MDA levels of patients with primary and metastatic liver cancers are higher than those in healthy control group.[7] In this study, MDA expression was particularly concentrated in much larger tumoral foci where pleomorphism predominated rather than in small tumoral foci. In parallel with these results, MDA expressions were more severe in neoplastic cells with hyperchromatic nuclei, increased number of nucleoli, and differentiated nucleus-to-cytoplasm ratios. In addition to these cells, the MDA staining index was much higher in tumoral cells with giant nuclei and necrotic foci. The MDA-positive staining was much more evident in macro- and micro-trabeculae and acinar formations. In addition to all these, MDA expression was stronger in macro-trabecular structures compared to the micro-trabecular structures.

When all these immunohistochemical findings are evaluated together, it is shown that oxidative stress and lipid peroxidation play an active role in the progression of HCC. Mizukami *et al.*[30] evaluated MDA expressions in the early stages of tumour promotion of DEN-induced liver cancer in rats using immunohistochemical methods and detected positive staining in the nuclei of hepatocytes. Contrary to this study, in the current investigation, MDA expressions were mostly granular and found in the cytoplasm of neoplastic liver cells. The difference between the staining patterns in these two studies is thought to be related to the lipid peroxidation causing nuclear damage. In the current study, only DEN was used for cancer modeling. In the other studies, other chemicals, such as phenobarbital, were used after DEN application. Depending on the severity of oxidative liver damage, staining may not have been limited to the cytoplasm but may have also been observed in the nucleus.

In conclusion, this study data contain three important findings. The first one is that DEN application triggers the ROS formation, excessively produced ROS cause oxidative stress, and as a result, oxidative stress strongly causes lipid peroxidation. Secondly, it is clear that there is an important relationship between oxidative stress-induced lipid peroxidation and HCC progression. At the same time, MDA is an useful biomarker in determining the prognosis of patients with HCC. The third and final finding is that intra-peritoneal DEN injection once a week for 20 weeks, but not in combination with other promoting chemical agents, appears to be very effective in inducing experimental HCC.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Ogar GO, Minari JB, Bello AJ, et al. Influence of ethanolic extract of *Allium sativum* on *TP53* gene and its anticancer potential in *N*-Nitrosodiethylamine (NDEA)-induced hepatocellular carcinoma in male albino rats. Iran J Basic Med Sci 2022; 25(4): 497-505.
2. Gerber TS, Witzel HR, Weinmann A, et al. Reduced lipid peroxidation predicts unfavorable prognosis in hepatocellular carcinoma, but not intrahepatic cholangiocarcinoma. Biomedicines 2023; 11(9): 2471. doi: 10.3390/biomedicines11092471.
3. Somade OT, Ajayi BO, Adeyi OE, et al. Ferulic acid interventions ameliorate NDEA-CCl_4-induced hepatocellular carcinoma via Nrf2 and p53 upregulation and Akt/PKB-NF-κB-TNF-α pathway downregulation in male Wistar rats. Toxicol Rep 2024; 12: 119-127.
4. Nassar-Reis JP, Umeta PF, Stefano JT, et al. P53 and VEGF are promising biomarkers for sorafenib efficacy in an experimental model of NASH-related HCC. J Mol Histol 2023; 54(5): 473-488.
5. Wang Z, Jiang X, Zhang L, et al. Protective effects of *Althaea officinalis* L. extract against *N*-diethylnitrosamine-induced hepatocellular carcinoma in male Wistar rats through antioxidative, anti-inflammatory, mitochondrial apoptosis and PI3K/Akt/mTOR signaling pathways. Food Sci Nutr 2023; 11(8): 4756-4772.
6. Chang W, Jiang XP, Jin S, et al. Synergistic effects of *CP-25* and 5-fluorouracil on the hepatocellular carcinoma in *vivo* and in *vitro* through regulating activating mitochondrial pathway. J Cancer 2022; 13(3): 1005-1018.
7. AboZaid OAR, Abdel-Maksoud MA, Saleh IA, et al. Targeting the NF-κB p65/Bcl-2 signaling pathway in hepatic cellular carcinoma using radiation assisted synthesis of zinc nanoparticles coated with naturally isolated gallic acid. Biomed Pharmacother 2024; 172: 116274. doi: 10.1016/j.biopha.2024.116274.
8. Hussein S, Soliman NA, Dahmy SIE, et al. Effectiveness of cannabidiol (CBD) on histopathological changes and gene expression in hepatocellular carcinoma (HCC) model in male rats: the role of Hedgehog (Hh) signaling pathway. Histochem Cell Biol 2024; 161(4): 337-343.
9. Karabekir SC, Özgörgülü A. Possible protective effects of resveratrol in hepatocellular carcinoma. Iran J Basic Med Sci 2020; 23(1): 71-78.
10. Unsal V, Kurutaş EB. Investigation of the effects of octreotide agent on oxidative stress, 8-hydroxy deoxyguanosine in experimental hepatic carcinogenesis rat model. Folia Med (Plovdiv) 2020; 62(1): 70-75.
11. Tawfik NG, Mohamed WR, Mahmoud HS, et al. Isatin counteracts diethylnitrosamine/2-acetylaminofluorene-induced hepatocarcinogenesis in male Wistar rats by upregulating anti-inflammatory, antioxidant, and detoxification pathways. Antioxidants (Basel) 2022; 11(4): 699. doi: 10.3390/antiox11040699.
12. You Y, Zhu F, Li Z, et al. Phyllanthin prevents diethylnitrosamine (DEN) induced liver carcinogenesis in rats and induces apoptotic cell death in HepG2 cells. Biomed Pharmacother 2021; 137: 111335. doi: 10.1016/j.biopha.2021.111335.
13. Fahim SA, Ibrahim S, Tadros SA, et al. Protective effects of butylated hydroxytoluene on the initiation of *N*-

nitrosodiethylamine-induced hepatocellular carcinoma in albino rats. Hum Exp Toxicol 2023; 42: 960327123 1165664. doi: 10.1177/09603271231165664.
14. Jin X, Zhao T, Shi D, et al. Protective role of fucoxanthin in diethylnitrosamine-induced hepatocarcinogenesis in experimental adult rats. Drug Dev Res 2019; 80(2): 209-217.
15. Aljuhr SA, Abdelaziz G, Essa BM, et al. Hepatoprotective, antioxidant and anti-inflammatory potentials of Vit-E/C@SeNPs in rats: synthesis, characterization, biochemical, radio-biodistribution, molecular and histopathological studies. Bioorg Chem 2021; 117: 105412. doi: 10.1016/j.bioorg. 2021.105412.
16. Bilal N, Suhail N, Hasan S, et al. Exacerbation of *N*-nitrosodiethylamine induced hepatotoxicity and DNA damage in mice exposed to chronic unpredictable stress. Front Pharmacol 2017; 8: 360. doi: 10.3389/ fphar.2017.00360.
17. Abouzed TK, Althobaiti F, Omran AF, et al. The chemoprevention of spirulina platensis and garlic against diethylnitrosamine induced liver cancer in rats via amelioration of inflammatory cytokines expression and oxidative stress. Toxicol Res (Camb) 2021; 11(1): 22-31.
18. Kim H, Hong MK, Choi H, et al. Chemopreventive effects of korean red ginseng extract on rat hepatocarcinogenesis. J Cancer 2015; 6(1): 1-8.
19. Gupta P, Bansal MP, Koul A. Lycopene modulates initiation of *N*-nitrosodiethylamine induced hepatocarcinogenesis: studies on chromosomal abnormalities, membrane fluidity and antioxidant defense system. Chem Biol Interact 2013; 206(2): 364-374.
20. Singh BN, Singh BR, Sarma BK, et al. Potential chemoprevention of *N*-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from Acacia nilotica bark. Chem Biol Interact 2009; 181(1): 20-28.
21. Fu Y, Chung FL. Oxidative stress and hepato-carcinogenesis. Hepatoma Res 2018; 4: 39. doi: 10.20517/2394-5079.2018.29.
22. Lorente L, Rodriguez ST, Sanz P, et al. Association between pre-transplant serum malondialdehyde levels and survival one year after liver transplantation for hepatocellular carcinoma. Int J Mol Sci 2016; 17(4): 500. doi: 10.3390/ijms17040500.
23. Elbaz S, Mousa N, Besheer T, et al. Malondialdehyde and C-reactive protein as prognostic markers of hepatocellular carcinoma. Br J Biomed Sci 2020; 77(2): 94-96.
24. Ramakrishnan G, Augustine TA, Jagan S, et al. Effect of silymarin on *N*-nitrosodiethylamine induced hepatocarcinogenesis in rats. Exp Oncol 2007; 29(1): 39-44.
25. Karakurt E, Dağ S. Protective effect of silymarin on diethylnitrosamine induced hepatocellular carcinoma model in Wistar rats; cancer patterns and cytological variants. Atatürk University J Vet Sci 2020; 15(1): 43-50.
26. Karakurt E, Dağ S. Hepatoprotective effect of silymarin against diethylnitrosamine induced oxidative and nitrosative stress in rats. Van Vet J 2020; 31(2): 105-110.
27. Karakurt E, Nuhoğlu H, Dağ S, et al. Immuno-histochemical investigation of TNF-α and IFN-γ expressions in sheep fetuses with brucellosis. Pak Vet J 2023; 43(1): 85-90.
28. Jayakumar S, Madankumar A, Asokkumar S, et al. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Mol Cell Biochem 2012; 360(1-2): 51-60.
29. Vanli S, Kurtoglu F, Alan BS, et al. Investigation of the effects of Theranekron and Sorafenib treatments on carcinogenesis, apoptosis and biochemical profile in hepatocellular carcinoma in rats. Toxicol Mech Methods 2024; 34(7): 750-760.
30. Mizukami S, Ichimura R, Kemmochi S, et al. Induction of GST-P-positive proliferative lesions facilitating lipid peroxidation with possible involvement of transferrin receptor up-regulation and ceruloplasmin down-regulation from the early stage of liver tumor promotion in rats. Arch Toxicol 2010; 84(4): 319-331.