Experimental Hepatic Carcinogenesis: Oxidative Stress and Natural Antioxidants

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Abstract

Hepatocellular carcinoma is one of the most common cancers in the world, and it is influenced by agents such as DEN, 2-AAF, phenobarbital, alcohol, aflatoxin B1 metabolite or hepatitis viruses (B and C). Oxidative stress is becoming recognized as a key factor in the progression of hepatocarcinogenesis. Reactive oxygen species can play a leading role in initiation and promotion of hepatic carcinogenesis. The metabolites of DEN Diethylnitrosamine (DEN) mediate the binding of tumour promoters by covalently binding to the DNA with one or two oxidation-providing electrons. 2-AAF is the inducer of DEN, and it is involved in tumour formation in the bladder and liver. Reactive Oxygen species (ROS); carbohydrates, lipids, DNA and enzymes, such as affect all important structures. Additionally, an excessive amount of ROS is highly toxic to cells. Antioxidants are protectors against ROS, toxic substances, carcinogens. This review focuses on the literature on studies of Hepatic Carcinogenesis, oxidative stress and antioxidant therapy.

Introduction

Hepatocellular carcinoma is the fifth most common cancer which third most common cause of cancer-related death globally and it is influenced by agents such as DEN, 2-AAF, phenobarbital (PB), alcohol, aflatoxin B1 metabolite or hepatitis viruses (B and C) [1-2].

Animal models are viewed as crucial tools in the study of hepatic carcinogenesis. Because of the physiologic and genetic similarities between rodents and humans, the short lifespan, the breeding capacity and the variety of manipulating methods, animal models are often used for cancer research [3]. Studies on induction of liver cancer in rats use chemical agents such as DEN, 2-AAF, PB and aflatoxin B1 [4].

2-AAF exhibits its carcinogenic effect through the formation of DNA adducts, over production of reactive oxygen species (ROS) and oxidative DNA damage [5]. Nitrosamines are widely recognized as carcinogenic compounds, but they require metabolic activation to exert their cytotoxic and carcinogenic activity. DEN is a nitrosamine compound that induces the formation of hepatic carcinoma. They showed that DEN increased lipid peroxidation in studies performed. This may increase the tumour [6-7].

Our aim in this study is to reveal the relationship between antioxidants and oxidative stress in experimental hepatic carcinogenesis studies. And to report chemopreventive natural antioxidants used as inhibitors.
Reactive oxygen species (ROS)

Reactive oxygen radicals; (O$_2$•$^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH•), which are present in small quantities during normal oxygen metabolism. Molecular oxygen (O$_2$) has two unpaired (unpaired) electrons with parallel spin states. An atom, group of atoms or molecules containing an unpaired electron, are defined as free radicals. Transition metals such as Fe$^{3+}$, Cu$^{2+}$, Mn$^{2+}$ and Mo$^{5+}$, however, are not considered free radicals even if they have unpaired electrons. But they play an important role in the formation of free radicals [8]. They are lipids that are most affected by reactive oxygen species. Since cell membranes are rich in polyunsaturated fatty acids (PUFAs) and cholesterol, they are easily affected by oxidant radicals. Lipid peroxidation, where the unsaturated lipids are present, is a complex process that takes place with reactions involving molecular oxygen and is formed by lipid hydroperoxides. Lipid peroxidation is rather harmful as it is an autocatalytic and irreversible reaction [9-10]. Lipid peroxidation produces a wide variety of oxidation products, such as malondialdehyde (MDA), propanal, hexanal, and 4-hydroxy nonanal (4-HNE). MDA appears to be the most mutagenic product of lipid peroxidation, but 4-HNE is most toxic. MDA is one of the most popular and reliable markers that determine oxidative stress in research [11]. Proteins are less sensitive to the effects of radicals than lipids. Protein oxidation results in the covalent modification of peptide bonds or amino acid side chains with ROS or oxidative stress products. In particular, the interaction of free radicals with unsaturated bonds and sulphide inclusion molecules is excessive. ROS may have direct or indirect effects on proteins. Amino acids such as peptide bonds, proline and lysine are quite easily affected by free radicals [12].

Protein oxidation occurs in the formation of carbonyl groups in amino acids such as histidine, tyrosine, phenylalanine. Products made by lipid peroxidation form covalent bonds with cysteine sulphydryl groups or with lysine and histidines, leading to fragments and cross-linking of proteins. These events result in the deterioration of the structure and function of the proteins. Protein carbonyl levels area well-used marker for oxidative stress. [8-15].

DNA Damage and Free Radicals

The DNA molecule can undergo spontaneous chemical oxidative damage like carbohydrates and proteins. It has been suggested that every cell DNA of the human body is exposed to oxidative damage 10$^3$ times a day [8]. Due to the balance between DNA damage and repair, very low levels of damage are also found in healthy individuals. Oxidative base modification (8-OHdG) has been shown even in newborn rats [16]. All changes that occur due to the effects of endogenous or exogenous factors in molecular integration are called DNA damage. 8-OHdG indicates DNA damage [17-18]. In recent years, base damage has frequently been analysed as an indicator of oxidative DNA damage. Since Cu$^{2+}$ ions are highly localized in the regions rich in G-C in DNA, the oxidative damage is the most exposed base guanine. 8-OHdG is a mutation that occurs in DNA, resulting from reactive oxygen species produced during normal oxidative metabolism [19]. All of the factors that lead to increased ROS production contribute to the formation of 8-OHdG, that is, oxidative DNA damage. The formation of 8-OHdG by substances such as cigarette smoke, x-rays, oxidized unsaturated fatty acids, gamma rays, polyphenols, paraquat, kainic acid, diethyl butyl sterol, benzene, fecapenene, furocoumarins hydroperoxide and heavy metals has been shown in vitro [20]. For example, Cigarette smoke contains carcinogenic substances such as nitrosamines and polycyclic aromatic hydrocarbons and causes the increased of 8-hydroxydeoxyguanosine [19]. For this reason, the most commonly measured base damage is 8-OHdG. Therefore, 8-OHdG is considered as the "biological marker" of DNA damage [17]. Increased ROS production and oxidative DNA damage associated with hepatocarcinogenesis have been demonstrated in studies. Multivariate analysis found that levels of 8-OHdG and fibrosis were significant risk factor for hepatocellular carcinoma, especially in patients with hepatitis C virus infection [21]. The marker of oxidative stress, such as 8-OHdG is commonly elevated in the livers of patients with chronic viral hepatitis infection, which is known to be a risk factor for HC.

Experimental Hepatic Carcinogenesis

Hepatic carcinogenesis can be created experimentally in experimental animals by the application of various chemicals such as aromatic amines, nitrogen containing dyes, nitrosamines and aflatoxins [22]. Xenobiotics are carcinogenic to animals such as DEN, 2-AAF and phenobarbital, mouse, rat, hamster, rabbit, dog, pig and monkey. DEN and 2-AAF are the chemicals that cause tumours to form in the biological system. [23-24-25]. Co-administration of DEN and 2-AAF initiates hepatocarcinogenesis in rodents and causes preneoplastic initiation in hepatocytes. [26-27-28]. In a study with FB acting as a promoter, such as DEN and 2-AAF, it was reported that they caused a mutation in codon 61 of H-Ras [29].

![Figure 1: DEN and 2-AAF of structure](http://www.mjms.mk/press.eu/mjms/)

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**Role of DEN in tumorigenesis**

DEN is mostly used as tumour inducer in cancer researches [30-31]. In the structure of DEN; Amide, urease and carbon containing compounds are available [32]. It has been reported that DENs are composed of intoxicates, from agrochemicals and nitratran, from those in cigarette smoke, as well as the formation of nutrients and nutrient nitrates [27-33]. DEN has a direct effect on cancer formation. This means that DEN spontaneously hydrolyzes, regardless of the enzymes. This biological activation of the active DEN by two hydroxylation reactions is catalysed by cytochrome p450. One strong mechanistic link between cancer is through the increased production of free radicals at the site of the resulting molecular changes, which include lipid peroxidation and oxidative DNA damage [34-35].

**The role of 2-AAF in tumorigenesis.**

2-AAF occurs as a result of the acetylation of the 2-amino florin in the synthetic arylamine structure. 2-AAF acts in the second phase of the detoxification reactions and after the first step of DEN, it binds to guanine base for the second time in DNA and creates a toxic effect. This toxic effect occurs in the form of preneoplastic, neoplastic and malignant neoplasms, respectively, resulting in mutations [36-39]. If the levels of DEN and 2-AAF chemical tumour inducing agents increase in cells, the smooth endoplasmic reticulum enzymes are synthesized and detoxified [40-42].

**Antioxidant Systems Against Reactive oxygen Species**

In the cells and extracellular fluid there are antioxidant defence mechanisms that try to bring the reactive oxygen radicals to a harmless state.

**Antioxidant enzyme systems, which convert ROS into less toxic products:** Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase enzymes (such as glutathione peroxidase (GSH-Px), glutathione reductase, etc.). SOD enzymatically converts superoxide anion to hydrogen peroxide and molecular oxygen. Hydrogen peroxide is reduced by water and oxygen with two important intracellular enzymes, catalase and glutathione peroxidase [43-46].

**Antioxidants that catch and neutralize the radicals:** Alpha Tocopherol (E vitamin) and Ascorbic acid (C vitamin) function as antioxidants. Vitamin E prevents lipid peroxidation in the cell membrane. Ascorbic acid shows antioxidant activity in the cytoplasm and extracellular fluids and inhibits the inactivation of antiproteases with oxidants. Additionally, Glutathione is a multifunctional intracellular antioxidant, α-Lipoic acid (ALA), which is a sulfur-containing antioxidant with metal-chelating and antiglycation capabilities. N-acetyl-L-cysteine is a thiol containing an antioxidant that has been used to decrease conditions of oxidative stress. The most reported activity of flavonoids is protection against oxidative stress. Thus flavonoids can help scavenger ROS and are effective inhibitors of lipid peroxidation [47].

**Systems that prevent the formation of ROS and prevent the formation of ROS**

Structures such as ceruloplasmin, ferritin, transferrin, lactoferrin, zinc, selenium, cytochrome oxidase reduce ROS. For example; Zinc has been serving as a metal that prevents lipid peroxidation and DNA damage [48-49].

**Experimental Investigations**

Most of the factors that influence tumour formation cause radical production in the cell. These factors also induce tumour formation and development by affecting the initiation, development and progression stages of carcinogenesis. Various animal model studies have been done on this subject. As seen in Table 1, many natural antioxidants have been tried.

| Models | Animals | Materials | Effect | Dose | References |
|--------|---------|-----------|--------|------|------------|
| DEN (200 mg/kg) | Wistar albino rats | Tannic acid | GSH↑, SOD↑, MDA↓ | 125 and 250 mg/kg | [51] |
| 2-AAF (200 mg/kg) | Rattus norvegicus | Tocotrienol | GSH↑ | 30 mg/kg | [54] |
| DEN (200 mg/kg) | Wistar albino rats | Thymoquinone | GSH↑, GSH↑ | 15 mg/kg | [56] |

**Materials**

- Cell lines: Rb1 melanoma, NIH3T3, Hep G2, THP-1, Raji, Caco-2, MCF-7, B16F10, A549, HT29, Hep-2, Hela, Jurkat, U937, MDM2, MDA-MB-231, MDA-MB-468, and 293T
- Enzymes: SOD, CAT, GPx, GST, and LPO
- Antioxidant compounds: NAC, Ascorbic acid, α-Lipoic acid, L-cysteine, BHT, and butylated hydroxyanisole (BHA)

**References**

- [30-31]
- [32]
- [27]
- [33]
- [55]
- [56]
- [57]
In conclusion, the relationship between HC and oxidative stress is a research area. ROS contributes to the initiation and progression of HC. In current clinical trials, the mechanisms of HC treatment of drugs or compounds may be partly due to antioxidant ability, especially the effect originating from ROS. Therefore, antioxidant therapeutics play an important role in the treatment of HC. Time, effective doses and reliable doses require further investigation of antioxidant absorption and bioavailability.

References

1. Kuroda HI, Ohtsuru AK, Futakuchi MI, Kawashita YU, Nagayama YU, Fukuda E, Namba HI, Shirai TO, Kanematsu TA, Yamashita SH. Distinctive gene expression of receptor-type tyrosine kinase families during rat hepatocarcinogenesis. International journal of molecular medicine. 2002;9(5):473-80. https://doi.org/10.3892/ijmm.9.5.473

2. Wood GA, Sarma DS, Archer MC. Inheritance of resistance to the promotion of preneoplastic liver lesions in Copenhagen rats. Experimental Biology and Medicine. 2001; 226(8): 831-835. https://doi.org/10.1177/153537020122600904 PMid:11568305

3. He L, Tian DA, Li PY, He XX. Mouse models of liver cancer: Progress and recommendations. Oncotarget. 2015; 6(27): 23306. https://doi.org/10.18632/oncotarget.4202 PMid:26259234

4. Itze L, Vesselinovitch SD, Rao KV. Estimation of the rate of DNA synthesis in newborn, regenerating and intact mice livers. Physiologia Bohemoslovaca. 1972; 22(5): 457-460.

5. Hasan SK, Sultana S, Geranial attenuates 2-acetylaminofluorene induced oxidative stress, inflammation and apoptosis in the liver of wistar rats. Toxicology Mechanisms and Methods. 2015; 25(7):559-573. PMid:26364502

6. Magee PN, Barnes JM. The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. British journal of cancer. 1956; 10(1):114. https://doi.org/10.1038-bjc.1956.15 PMid:13342328

7. Yamada KI, Yamamiya I, Utsumi H. In vivo detection of free radicals induced by diethylnitrosamine in rat liver tissue. Free Radical Biology and Medicine. 2008; 40(11):2040-204. https://doi.org/10.1016/j.freeradbiomed.2006.01.031 PMid:16716904

8. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford University Press, USA, 2015. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001

9. Arican O, Ozuturk P, Korutas EB, Unsal V. Status of oxidative stress on lesional skin surface of plantar warts. Journal of the European Academy of Dermatology and Venereology. 2003; 27(3):365-369. https://doi.org/10.1111/j.1468-3083.2003.01419.x PMid:12222120

10. Slater TF. Overview of methods used for detecting lipid peroxidation. Methods in enzymology. 1984; 105:283-293. https://doi.org/10.1007/S0076-6789(84)05036-9

11. Esterbauer H, Eckl P, Ortner A. Possible mutagens derived from lipids and lipid precursors. Mutation Research/Reviews in Genetic Toxicology. 1990; 238(3):223-233. https://doi.org/10.1016/0165-1101(90)80014-3

12. Stadtmann ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino acids. 2003;25(3-4): 207-218. https://doi.org/10.1007/s00726-003-0011-2 PMid:14661084

13. Suzuki YJ, Carini M, Butterfield DA. Protein carbonylation. Antioxidants & redox signaling. 2010; 12(3): 323-325. https://doi.org/10.1089/ars.2008.2887 PMid:19743917 PMcid:PMC2821144

14. Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Mitlani A. Protein carbonylation in human diseases. Trends in molecular medicine. 2003; 9(4):169-176. https://doi.org/10.1016/S1471-4914(03)00031-5

15. Kehler JP, Smith CV. Free radical in biology: Sources, reactivities and roles in the etiology of human disease. Natural Antioxidants in Human Health and Disease. Academic Press, San Diego, 1994:25-62.

16. Randerath K, Zhou GD, Monk SA, Randerath E. Enhanced levels in neonatal rat liver of 7, 8-dihydo-8-oxo-2′-deoxyguanosine (8-hydroxydeoxyguanosine), a major mutagenic oxidative DNA lesion. Carcinogenesis. 1997; 18(7):1419-21. https://doi.org/10.1093/carcin/18.7.1419 PMid:9230290

17. Cooke MS, Evans MD, Dizdaroglu M, Lunej J. Oxidative DNA damage: mechanisms, mutation, and disease. The FASEB Journal. 2003; 17(10):1195-1214. https://doi.org/10.1096/fasebj.02-075rev PMid:12832285

18. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez, H. Free radical-induced damage to DNA: mechanisms and measurement 1, 2. Free Radical Biology and Medicine. 2002;32(11):1102-1115. https://doi.org/10.1016/S0891-5849(02)00826-2

19. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. Journal of carcinogenesis. 2006; 5(1): 14. https://doi.org/10.1186/1475-2861-5-14 PMid:1668993 PMcid:PMC1479806

20. Shoji Fukushima MG, Kakehashi A, Wanibuchi H. Qualitative and Quantitative Assessments on Low-Dose Carcinogenicity of Genotoxic Carcinogens: Dose—Response for Key Events in Rat Hepatocarcinogenesis. Thresholds of Genotoxic Carcinogens: From Mechanisms to Regulation, 2016.

21. Chuma M, Hige S, Nakanihshi M, Ogawa K, Natsuizaka M, Yamamoto Y, Asaka M. 8-Hydroxy-2′-deoxyguanosine is a risk factor for development of hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Journal of gastroenterology and hepatology. 2008; 23(9):1431-1436. https://doi.org/10.1111/j.1440-1746.2008.05502.x PMid:18854000

22. Glauert HP, Calfee-Mason K, Steinn DN, Tharrapel JC, Spear BT. Dietary antioxidants in the prevention of hepatocarcinogenesis: a review. Molecular nutrition & food research. 2010; 54(7):875-896. https://doi.org/10.1002/mnr.20512789

23. Ahn B, Han BS, Kim DJ, Ohshima H. Immunohistochemical Localization of Inducible Nitric Oxide Synthase And 3-Nitrotyrosine in Rat Liver Tumors Induced by N-Nitrosodimethylamine. Carcinogenesis. 1999; 20(7): 1337-1344. https://doi.org/10.1093/carcin/20.7.1337 PMid:10383909

24. Barbason H, Mormont C, Massart S, Bouzahzah, B. Anti-carcinogenic action of phenobarbital given simultaneously with diethylnitrosamine in the rat. European Journal of Cancer and Clinical Oncology. 1986; 22(9):1073-1078. https://doi.org/10.1016/0277-5379(86)90008-8

25. Kowsalya R, Kailaperumal J, Vaishnavi M, Namasivayam E. Anticancer activity of Cynodon dactylon L. root extract against diethylnitrosamine induced hepatic carcinoma. South Asian Journal of Cancer. 2015; 4(2):83-88. https://doi.org/10.4103/2278-330X.155691 PMid:25992348 PMcid:PMC4418089

26. Thirunavukkarasu C, Sakhishekar D. Stabilization of membrane bound enzyme profiles by sodium selenite in Nitrosodimethylamine induced and phenobarbital promoted hepatocarcinogenesis in rats. Biomedicine & pharmacotherapy. 2003; 57(3):117-123. https://doi.org/10.1016/S0753-3322(03)00016-7

27. Bingül İ, Başaran-Küçükgergin C, Tekkeşin MS, Olgaç V, Doğru-Abbasoglu S, Uysal M. Effect of blueberry pretreatment on diethylnitrosamine-induced oxidative stress and liver injury in rats. Environmental toxicology and pharmacology. 2013;36(2):529-538. https://doi.org/10.1016/j.etap.2013.05.014 PMid:2381110
function for erythrocrepin (hemocuprein). Journal of Biological chemistry. 1969;244(22):6049-6055. PMid:5389100

44. Roos DIRK, Weening RS, Wyss SR, Aebi HE. Protection of human neutrophils by endogenous catalase: studies with cells from catalase-deficient individuals. Journal of Clinical Investigation. 1980; 65(6):1515. https://doi.org/10.1172/JCI109817 PMid:7410555 PMCID:PMC371491

45. Halliswell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? The lancet. 1994; 344(8924):721-724. https://doi.org/10.1016/S0140-6736(94)92211-X

46. Aydogan F, Unlu I, Aydin E, Yumusak N, Devrim E, Samim EE, Seyhan N. The effect of 2100 MHz radiofrequency radiation of a 3G mobile phone on the parotid gland of rats. American journal of otolaryngology. 2015; 36(1):38-46. https://doi.org/10.1016/j.amjoto.2014.10.001 PMid:25456509

47. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition. 2016;15(1): 77. https://doi.org/10.1186/s12937-016-0186-5 PMid:27456681 PMCID:PMC4960740

48. Silva DD, Aust SD. Ferritin and ceruloplasmin in oxidative damage: review and recent findings. Canadian journal of physiology and pharmacology. 1993; 71(8):715-720. https://doi.org/10.1139/y93-107

49. Unsul V, Kurutas EB, Öztürk P. The effects of the H1-receptor blockers on Adenosine Deaminase, xanthine oxidase and Trace elements in patients with chronic idiopathic Urticaria. European Journal of Pharmacological and Medical Research. 2017;4(3):117-121.

50. Abdel-Hamid NM, Mohafez OM, Nazmy MH, Farhan A, Thabet K. The effect of co-administration of Lawsonia inermis extract and octreotide on experimental hepatocellular carcinoma. Environmental health and preventive medicine. 2015; 20(3):195-203. https://doi.org/10.1007/s12119-015-0451-9 PMid:25726025 PMCID:PMC4432434

51. Sehrawat A, Sharma S, Sultana S. Preventive effect of tannic acid on 2-acetylaminofluorene induced antioxidant level, tumor promotion and hepatotoxicity: a chemopreventive study. Redox Report. 2006; 11(2):85-95. https://doi.org/10.1179/135100006X101066 PMid:1666999

52. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, Rajeshwar Y, Saravanan S, Kumar MS, Muruges K, Kumar KA. Antioxidant and antimicrobial activities of Bauhinia racemosa L. stem bark. Brazilian journal of medical and biological research. 2003;36(7):1015-24. https://doi.org/10.1590/S0100-879x2000000700004 PMid:16070272

53. Farombi EO, Tahteng JG, Agboola AO, Nwankwo JO, Emerole GO. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kola—Garcinia kola seed extract. Food and Chemical Toxicology. 2000;38(6):535-41. https://doi.org/10.1016/S0278-6915(00)00339-9

54. Rahmat A, Ngah WZ, Shamaan NA, Gapor A, Abdul KK. Long-term administration of tocotrienols and tumor-marker enzyme activities during hepatocarcinogenesis in rats. Nutrition (Burbank, Los Angeles County, Calif.). 1993;9(3):229-32. PMids:8102564

55. Sayed-Ahmed MM, Aleisa AM, Al-Rejaie SS, Al-Yshaya AA, Al-Shabany OA, Hafez MM, & Nagi MN. Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. Oxidative medicine and cellular longevity. 2010; 3(4):254-261. https://doi.org/10.14161/oxim.3.4.12714 PMid:20972371 PMCID:PMC2952085

56. Jayakumar S, Madankumar A, Asokkumar S, Raghunandhakumar S, Kamaraj S, Divya MGJ, Devaki T. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Molecular and cellular biochemistry. 2012; 360(1-2):51-60. https://doi.org/10.1007/s11010-011-1043-7 PMid:21879312

57. Pradeep K, Mohan CVR, Gobianand K, Karthikeyan S. Effect of Unsal & Kurutas. Experimental Hepatic Carcinogenesis: Oxidative Stress and Natural Antioxidants.
Cassia fistula Linn. leaf extract on diethylnitrosamine induced hepatic injury in rats. Chemico-Biological Interactions. 2007; 167(1):12-18. https://doi.org/10.1016/j.cbi.2006.12.011 PMid:17289008

58. Husain Khan T, Sultana S. (2011). Effect of Aegle marmelos on DEN initiated and 2-AAF promoted hepatocarcinogenesis: a chemopreventive study. Toxicology mechanisms and methods. 2011; 21(6):453-462. https://doi.org/10.3109/15376516.2011.564677 PMid:21417629

59. Sun C, Kono H, Furuya S, Hara M, Hirayama K, Akazawa Y, Nakata Y, Fujii H. Interleukin-17A plays a pivotal role in chemically induced hepatocellular carcinoma in mice. Digestive diseases and sciences. 2016;61(2):474-88. https://doi.org/10.1007/s10620-015-3888-1 PMid:26467699

60. Hassan SK, Mousa AM, Eshak MG, Farrag AERH, Badawi AEFM. (2014). Therapeutic and chemopreventive effects of nano curcumin against diethylnitrosamine induced hepatocellular carcinoma in rats. Int J Pharm Pharm Sci. 2014; 6(3):54.