Oxidative stress predominates apoptosis during experimental hepatocellular carcinoma

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Objective Hepatocellular carcinoma ranks the third among cancer deaths in the globe. Despite the fact that many strategies for diagnosing liver cancer are possible, many factors, including apoptosis, arise to interfere with cell cycle control. We initiated this study to investigate the importance of free radicals during experimental hepatocarcinogenesis, triggered by diethyl nitrosamine, against an anti-apoptotic factor, belongs to a family of oncogenes (Bcl-2).
Methods Eighteen female Wistar rats were classified into two equal groups, group 1 received intraperitoneal normal saline doses, group 2 was injected with one IP dose of diethyl nitrosamine (200 mg/kg bw), 2 weeks later, they were given a single CCl_4 dose (2 ml /kg b.w., IP), blood and liver tissue samples were collected after 60 days of diethyl nitrosamine dose. The Graph pad prism program was used in statistical calculations.
Results The results revealed that treatment with diethyl nitrosamine + CCl_4 significantly up regulated the plasma liver functions tests: aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, total bilirubin and increased alpha fetoprotein with decreased level of Bcl-2. Liver tissue showed elevation of lipid peroxide, malondialdehyde, catalase enzyme activity, with significant reduction in body weight, decreased total protein content, tissue antioxidants like glutathione *S*-transferase, total thiol and total antioxidant capacity, compared to control. Histological examination of liver showed an increased nuclear/cytoplasm (N/C) ratio, mitotic figures and nuclear polymorphism and microacinar formation in diethyl nitrosamine + CCl_4-treated group.
Conclusions Disturbed hepatocellular anti-oxidant mechanisms suppress apoptosis, reflecting failure in combating chemical hepatocarcinogenesis.
Keywords liver cancer; diethyl nitrosamine; lipid peroxidation; tumor markers; Bcl-2; apoptosis.

Introduction

Globally, hepatocellular carcinoma (HCC) ranks the sixth prevalent cancer and the third cause of death related to cancer.[1] The well-known risk factors of HCC include untreated viral hepatitis (HBV and HCV), some food flavors, alcohols, aflatoxins, toxic chemicals from industrial processes and environmental pollutants.[2] Diethyl nitrosamine (DENA), a strong hepatocarcinogen, was reported induce aberrations in the nuclear enzymes engaged in DNA repair and replication.[3] Nitrate and nitrite are used as preservatives in both meat and fish industry, color fixatives and cost effective flavors that can generate endogenous hepatotoxic nitroso byproducts, like primary amines in the stomach pH.[4] Metabolic activation of DENA yields pro-mutagenic adducts, O6-ethyl deoxy guanosine and 04 and O6-ethyl deoxy thymidine in liver, leading to hepatocarcinogenesis.[5] DENA liver model cancer is one of the most commonly used experimental models in hepatocarcinogenesis.[6]

It is now documented that interference to apoptosis is a contributor to HCC.[7] This was also proved by oxidative stress,[8] as well as, the impact of oxidative stress on apoptotic pathways was reported.[8,9] One approach to control liver cancer, is studying the different factors underline the progression of the disease. Oxidative stress is an important risk factors contributing to HCC and other cancers.[10] B cell lymphoma protein-2 (Bcl-2) is a survival protein, found in outer mitochondrial membrane, known to suppress the release of cytochrome C from the mitochondria; so, it can interfere apoptosis in cancer cells, thus, it contributes in development of cancer and resistance to anticancer compounds.[11]

The aim of the current work was to assess the ability of reactive oxygen metabolites in disturbing apoptosis, through studying the impact on an oncogene(Bcl-2), during experimental HCC. This may lead to a more efficient strategy for treatment success via adjuvant therapeutics with a more selective antioxidant efficacy with the common or new therapeutic protocols of HCC. In addition to liver functions, we will estimate oxidative stress markers, as well as, an anti-apoptotic mediator (oncogenic protein Bcl-2), with AFP and histopathology of liver tissue.

Materials and Methods

Chemicals

DENA and the other fine chemicals were obtained from Sigma-Ahlrich Chemical Co., USA. Rat AFP and rat Bcl_2 Elisa chemicals from TSZ Scientific LLC, USA. Other used chemicals were of analytical grade, purchased from local suppliers.

Animals

We recruited 18 female rats weighing 180 g (±20 g) obtained from the Faculty of Veterinary Medicine, in the University of Cairo, Egypt. They were accommodated for 1 week before

experimental work on standard chow and drinking water in the laboratory of Department of Zoology, Faculty of Science, University of Tanta, Egypt. The temperature was kept at 23 ± 2°C with a approximate humidity of 60% under 12 h/12 h light dark cycle. Animals were classified as two equal groups (9 animals each).

Experimental Design

Group 1(G1): Control group, rats were given saline, intraperitoneally (i.p) for 60 days.

Group 2(G2): HCC group, rats were given one dose of diethylnitrosamine dissolved in saline (i.p) in the dose of 200 mg/kg b.w.[12] And after 2 weeks, they were given an activating dose of carbon tetrachloride (CCl_4) in olive oil in a dose of 2 ml /kg b.w. (i.p). Euthanization of rats was performed after 60 days from DENA injection.

Blood and Tissue Collection and Preparation

By the end of the experiment, animals were left without any chow overnight, weighted and euthanized. Blood was collected retro-orbitally by capillary tubes, left for 10 minutes, centrifuged at 3000 rpm for 10 min and sera were collected and kept in clean stoppered plastic vials at −80°C right analyses of alanine transaminase (ALT), AST, Gamma-glutamyl transferase (GGT), T. bilirubin, AFP and Bcl-2.

Both ALT and AST activities were spectrophotometrically estimated,[13] GGT activity was determined [14] and bilirubin was spectrophotometrically estimated.[15] Quantitative measurement of AFP level in serum was executed by ELISA kit (WKEA Med Supplies Corp, China, code no. WAR-348), following the manufacturers' instructions. Bcl-2 concentration was computed by commercial kit, following the insert instructions [Bcl-2 kit (biorbyt), Life science (USCNK) Company Inc UK Cat. No Orb52840], by sandwich enzyme immunoassay. The absorbance was measured by ELISA plate reader at 405 nm.[16]

Liver tissue was immediately isolated, cleaned from tissues adhering matters, washed by saline solution, cold by ice, then dried on a filter paper and frozen at −80°C. The liver tissues were homogenized in potassium phosphate buffer (10% W/V, 0.01 M pH 7.4) for estimation of glutathione *S*-transferase (GST), catalase (CAT) enzyme activities, total thiol (TT), total antioxidant capacity (TAC) and total protein (TP) content. KCl solution (1.15 M) was used for estimation of malondialdehyde (MDA) using homogenizer (Hettich model EBA 12R, Germany).

MDA is an end product, produced by decomposition of unsaturated fatty acids attacked by free radicals. MDA was measured spectrophotometrically.[17] The protein content in the tissues was determined spectrophotometrically.[18] TT was assayed by DTNB.[19] TAC was determined utilizing the ferric reducing antioxidant potential.[20] GST enzyme activity was assayed after formation of adduct, through coupling of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB), as described before.[21] CAT enzyme activity monitored depending on H_2O_2 decomposition at 240 nm.[22]

Histological evaluation

Histological study was applied on serial random liver sections (5-mm thick), using rotary microtome (Litz, Wetzlar, Germany) and were stained with Haematoxylin and Eosin (H&E) staining.[23]

Statistical analysis

The results were shown as mean ± SEM. Significance of data variations were assessed by one way analysis of variance (ANOVA), followed by computing *t*-test, which compare between the two groups, using Graph pad prism software.[24] A value of $P < 0.05$ was our margin of statistical significance.

Results

Table 1 depicted that the group treated with DENA depicted significant up regulation of ALT, AST and GGT activities in plasma ($P < 0.001$) compared to control. Total bilirubin and AFP levels of this group was significantly elevated ($P < 0.001$), in relation to control group showing the destructive role of DENA. Conversely, serum Bcl-2 level was significantly depressed ($P < 0.001$), compared to control. Table 2, which included liver tissue chemistry, showed that DENA injection significantly elevated MDA level ($P < 0.001$), in relation to control. Both TP ($P < 0.01$), total thiol ($P < 0.001$) contents, TAC ($P < 0.01$), GST activity ($P < 0.001$) and body weight ($P < 0.001$) were significantly decreased, while catalase activity was significantly upregulated ($P < 0.001$), compared to control.

Table 1. **Variations in serum ALT, AST, GGT enzyme activities, total bilirubin, AFP and BCl_2 levels in female albino rats treated with hepatocarcinogen (HCC group), compared to control (Values are expressed as mean ± SEM; number of rats = 9)**

Parameters Group	ALT (U/l)	AST (U/l)	GGT (U/l)	T. Bilirubin (mg/dl)	AFP (ng/ml)	BCl_2 (ng/ml)
Control	11.5 ± 0.3	93.7 ± 1.1	4.7 ± 0.3	0.172 ± 0.016	178 ± 8.2	458 ± 11.3
HCC	19.7 ± 0.7***	127 ± 2.9***	13.1 ± 0.4***	0.3 ± 0.02***	277 ± 10.9***	347 ± 9.07***

***, indicates significant, compared to control at $P < 0.001$; **, significant, compared to control at $P < 0.01$; *, significant, compared to control at $P < 0.05$.

Table 2. **Variations in the liver tissue content of MDA, total protein, total thiol, TAC,GST and CAT activities, body weight and relative liver weight in female albino rats treated with hepatocarcinogen (HCC group), compared to control (Values are expressed as mean ± SEM; number of rats = 9)**

Parameters Group	MDA (nmol/g)	Total protein (mg/g)	Total thiol (mM/g)	TAC (µmol/g)	GST (mol/min/g)	Catalase (mol/min/g)	Body weight (g)
Control	123.1 ± 11.5	91 ± 1.4	41.22 ± 0.80	13.6 ± 0.8	5.41 ± 0.069	2.07 ± 0.06	30.8 ± 1.2
HCC	230 ± 5.6***	84 ± 0.7*	30.1 ± 0.7***	9.7 ± 0.6**	4.0 ± 0.13***	2.9 ± 0.05***	12 ± 2.4***

***, indicates significant, compared to control at $P < 0.001$; **, significant, compared to control at $P < 0.01$; *, significant, compared to control at $P < 0.05$.

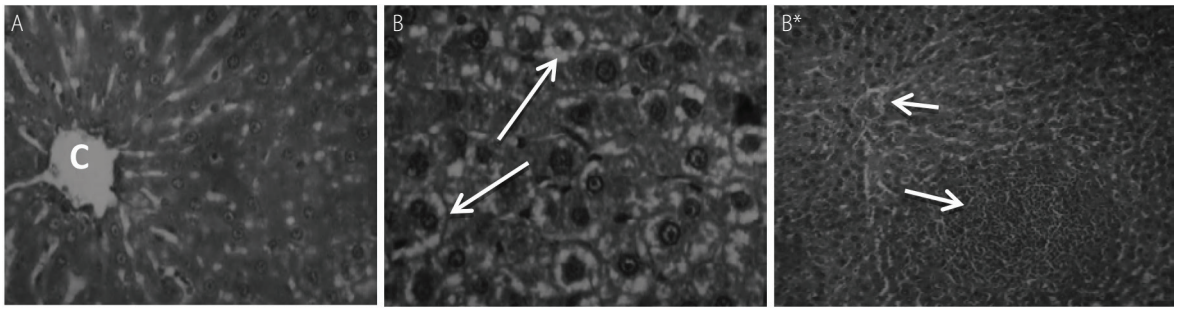

Fig. 1 **Histological investigation of H&E stained liver sections in control and HCC groups. (A) stands for photomicrographs of control group(X400), (B) and (B*) are micrographs of HCC group(X200, X100, respectively): N.B: A. Control hepatic tissue sections show normal cellular architecture. B: hepatic tissue section after DENA injection, shows increased nuclear/cytoplasm (N/C) ratio. B* Hepatic tissue section shows increased mitotic figures and nuclear polymorphism, increased width of cord cells, with microacinar formation.**

Histological evaluation of liver tissue from experimental groups after H&E staining showed that the control group had normal architecture, large polygonal cells with round nuclei and regular hepatic sinusoids arranged among hepatic cords (Fig. 1A). The surrounding hepatocytes showed pyknotic small peripheral nuclei and acidophilic vacuolated cytoplasm and binuclear cells were observed (Fig. 1B). Stained sections of the DENA-intoxicated group revealed a loss of the normal architecture of the liver, with congestion of the central veins and micronodules of varying sizes containing mononuclear inflammatory infiltration were also observed. Pigmented hyper plastic Kupffer cells were also seen (Fig. 1B*).

Discussion

The present observation was carried out to pursue the ability of free radicals to modulate apoptotic behavior in hepatocellular carcinogenesis in an experimental model. AST and alanine transaminase (ALT) are reliable markers for liver damage assessment. Hepatotoxicity disrupts hepatocytes membrane leading to spillage of transaminases into plasma.[25] GGT, shows tissue specific action and is up regulated during many normal and disease conditions, as development and carcinogenesis.[26]

Our study showed that DENA-intoxicated group, had a significant elevation in ALT, AST and GGT activities. This elevation was referred to the potential of DENA to release free radicals which damage cell membranes.[27] Plasma total bilirubin is a sensitive test for the assessment of liver diseases.[28] Plasma total bilirubin was significantly elevated in DENA-injected rats, possibly due to interference with the glucoronidation reaction and liberation of unconjugated bilirubin away from damaged liver tissue.[29,30] High levels of AFP are suggestive of HCC, and more than 70% of HCC holders show high plasma concentration due to tumor secretion. The up regulation of AFP level observed in DENA-treated animals is suggestive of HCC.[31] In our study, the anti-apoptotic factor, Bcl_2, in the group treated with DENA was significantly depressed. This was reported before in DENA related hepatic cancer, where, the initiation and progression of primary HCC was associated with proliferation and disturbed apoptosis linked to abnormal liberation of Bcl-2 and Bax genes.[32] We noticed that oxidative potential resulted from injection of DENA, manifested as, lipid peroxidation and perturbation of membrane unsaturated fatty acids in the cells might oppose apoptosis.

Oxygen-free radicals hit polyunsaturated fatty acid terminals in phospholipids. MDA is the famous end product of lipid peroxidation, which affect DNA forming chemical adducts. Lipid peroxidation contributes to endogenous DNA changes in humans leading to cancer and other related diseases.[33]

In this study, we found significant elevation in liver content of MDA in group treated with DENA. This was previously observed in liver and lung.[34]

Reactive oxygen species (ROS) generated in cells can damage cell membrane, leading to decrease the cellular protein synthetic function.[35] In our results, TP content in HCC group was significantly decreased compared to control, mostly a result of the cellular damage generated by DENA. This could be confirmed by previous reports.[36]

TT content of the body , including Sulfhydryl (SH) terminals found in protein are sought as major systemic antioxidant *in vivo*, carried by albumin.[37] They behave as reducing moieties in body compartments. Biosynthesis of both cysteine and glutathione mainly occurs in hepatocytes, however, the rest tissues get thiols through sinusoidal supply into blood, thus any damage in liver tissue will affect its production.[38]

In this study, the significant down regulation of the total non-protein thiols and protein thiols confirmed oxidative stress referred to generated electrophiles by DENA toxicity. This shows an accordance with.[39] TAC possesses superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), macromolecules as albumin, ceruloplasmin and ferritin. TAC constitutes the collective effect of all antioxidants in body compartments.[40] In the current work, TAC was decreased in group treated with DENA.

GST mediates the combination of GSH to many electrophilic compounds, as carcinogens, and native reactive species.[41] These compounds become less harmful than the original phase and are excreted out. In our experiments, we have observed a striking decrease in GST activity in liver after DENA treatment. This was reported earlier, where DENA administration initiated renal carcinogenesis with the same effect on GST activity.[36]

Catalase is a peroxisomal enzyme that mediates breakdown of H_2O_2 into O_2 and water. It plays a pivotal role in cellular oxidant protection. Fe-catalase is a tetrameric metalloprotein with protohaem, being the major structural component of the active site and the principal determinant of enzymatic activity.[42] (Print Our observations depicted that experimental HCC group contained a significant elevation of catalase activity. It seems that CAT activity was increased,

since the level of H_2O_2 is elevated in HCC group. Previous report depicted that few human cancer cell lines and tissues produced a high amount of H_2O_2 during cancer development.[39,43,44]

The results of our study indicated significant reduction in body weight gain (BWG) of the group treated with DENA, in respect to control group. This was in accordance with Naura et al.[34] who showed that DENA administration, greatly decreased anima total body weight. This was, in part, referred to the fact that, cancerous tissue and severe inflammation of hepatocytes may depress the muscle mass formation.[45]

Conclusion

We conclude that, disturbed antioxidant status, liberation of harmful-free radicals, concomitant to depressed cellular anti-oxidant potential may depress the apoptotic machinery, leaving a free tendency of hepatocytes to carcinogenesis. The elevated oxidative stress, shown by depressed antioxidant mediators plus elevated stress inducers, parallel to the decreased Bcl-2 level, points for assuming that, the oxidative stress contributes priorly role in hepatocarcinogenesis than the pro oncogenic Bcl-2. This promotes diagnosis and treatment of cancer, relying on the studied dilemma of variables, without ignoring oxidative stress at any stage.

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; ROS, reactive oxygen species; GST, glutathione-s-transferase; CAT, catalase; SH-groups, sulfhydryl groups; DENA, diethyl nitrosamine; TAC, total antioxidant capacity; MDA, malondialdehyde; T.Bil, total bilirubin; GGT, gamma glutamyl transferase; BWG, body weight gain, AFP, Alpha feto protein. Bcl-2, B cell lymphoma 2, TT, total thiol.

Author Contributions

Nabil contributed to conception, design, critical review of the manuscript, Afrah shared analysis and point concepts, mostafa shared drafting and review the manuscript, Naglaa drafted the manuscript and Asmaa executed the experimental work and drafted the article.

Declaration of Conflicting Interests

The authors declare that no conflicts of interest regarding publication of the manuscript.

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Ethical Approval

All implemented steps in this work complied with the ethical standards of the University Research Committee and with the Helsinki declaration; no formal ethical review was required.

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References

1. Theise, ND, Chen CJ, Kew MC. Liver Cancer; Lyon. 2014;577–593.
2. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer. 2006;6:674–687.
3. Bhosale PML, Ignle AD, Gadre RV, Rao KVK. Protective effect of Rhodotorula glutinis NCIM3353 on the development of hepatic preneoplastic lesions. Current Science. 2002;83:303–308.
4. Lin K, Shen ZY, Lu SH, Wu YN. Intake of volatile N-nitrosamines and their ability to exogenously synthesize in the diet of inhabitants from high-risk area of esophageal cancer in southern China. Biomed Environ Sci. 2002;15:277–282.
5. Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther. 1996;71:57–81.
6. Ha WS, Kim CK, Song SH, Kang CB. Study on mechanism of multistep hepatotumorigenesis in rat: development of hepatotumorigenesis. J Vet Sci. 2001;2:53–58.
7. Abdel-Hamid NM, Nazmy MH, Abdel-Ghany MI, Nazmy WH. Cytokines as important playmakers of experimental hepatocarcinogenesis confounded by diabetes. Ann Hepatol. 2012;11:118–127.
8. Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbecks Arch Surg. 2006;391:499–510.
9. Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med. 2000;29:323–333.
10. Abdel-Hamid NM. Recent insights on risk factors of hepatocellular carcinoma. World J Hepatol. 2009;1:3–7.
11. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35:495–516.
12. Al-Rejaie SS, Aleisa AM, Al-Yahya AA, Bakheet SA, Alsheikh A, Fatani AG, et al. Progression of diethylnitrosamine-induced hepatic carcinogenesis in carnitine-depleted rats. World J Gastroenterol. 2009;15:1373–1380.
13. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28:56–63.
14. Szasz G. A kinetic photometric method for serum gamma-glutamyl transpeptidase. Clin Chem. 1969;15:124–136.
15. Walter MGH. A colorimetric method for determination bilirubin in serum and plasma. Micro Chem J. 1970;15:231–236.
16. Sabokrouh A, Goodarzi MT, Vaisi-Raygani A, Khatami S, Taghizadeh-Jahed M. Effects of treatment with platinum azidothymidine and azidothymidine on telomerase activity and bcl-2 concentration in hepatocellular carcinoma-induced rats. Avicenna J Med Biotechnol. 2014;6:200–209.
17. Lahouel M, Boulkour S, Segueni N, Fillastre JP. [The flavonoids effect against vinblastine, cyclophosphamide and paracetamol toxicity by inhibition of lipid-peroxydation and increasing liver glutathione concentration]. Pathol Biol. 2004;52:314–322.
18. Ohnishi ST, Barr JK. A simplified method of quantitating protein using the biuret and phenol reagents. Anal Biochem. 1978;86:193–200.
19. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25:192–205.
20. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth Enzymol. 1999;299:15–27.
21. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249:7130–7139.
22. Xu JB, Yuan XF, Lang PZ. Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. Chin Environ Chem. 1997;16:73–76.
23. Bancroft JD, Cook HC. In Manual of Histological Techniques and their Diagnostic Application., Churchill Livingstone: Edinburgh, London, New York, Tokyo; 1994. pp. 23–26.
24. Graph P. Graph Pad Instate Soft Ware. www.graphpad.com.
25. Whittby LG, Perey-Robb IW, Smith AT. Enzymes tests in diagnosis. In Lecture Notes In Clinical Chemistry, 3rd eds, Black Well Scientific Publications; 1984. pp. 138–169.

26. Yao DF, Dong ZZ, Yao DB, Wu XH, Wu W, Qiu LW, et al. Abnormal expression of hepatoma-derived gamma-glutamyltransferase subtyping and its early alteration for carcinogenesis of hepatocytes. HBPD INT. 2004;3:564–570.
27. Jayakumar S, Madankumar A, Asokkumar S, Raghunandhakumar S, Gokula dhas K, Kamaraj S, et al. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Mol Cell Biochem. 2012;360:51–60.
28. Ramakrishnan G, Augustine TA, Jagan S, Vinodhkumar R, Devaki T. Effect of silymarin on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. Exp Oncol. 2007;29:39–44.
29. Rajkapoor B, Jayakar B, Murugesh N, Sakthisekaran D. Chemoprevention and cytotoxic effect of Bauhinia variegata against N-nitrosodiethylamine induced liver tumors and human cancer cell lines. J Ethnopharmacol. 2006;104:407–409.
30. Jagan S, Ramakrishnan G, Anandakumar P, Kamaraj S, Devaki T. Antiproliferative potential of gallic acid against diethylnitrosamine-induced rat hepatocellular carcinoma. Mol Cell Biochem. 2008;319:51–59.
31. Endo Y, Kanai K, Oda T, Mitamura K, Iino S. Clinical significance of alpha-fetoprotein in hepatitis and liver cirrhosis. Ann N Y Acad Sci. 1975;259:234–238.
32. Liu H, Zhang L, Chen S, Wang Z, Huang, F, Wang D. The role and significance of Bcl-2 and Bax in the hepatic carcinoma. Int J Morphol. 2012;30: 1466-1473.
33. Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. Toxicology. 2002;181–182:219–222.
34. Naura AS, Kalla NR, Sharma RP, Sharma R. Anticarcinogenic effects of hexaamminecobalt(III) chloride in mice initiated with diethylnitrosamine. Biol Trace Elem Res. 2007;119:147–165.
35. Abdel-Wahhab MA, Omara EA, Abdel-Galil MM, Hassan NS, Nada SA, Saeed A, et al. Zizyphus spina-christi extract protects against aflatoxin B1-initiated hepatic carcinogenicity. Afr J Tradit Complement Altern Med. 2007;4:248–256.
36. Pracheta SV. Anti-carcinogenic potential of Euphorbia neriifolia leaves and isolated flavonoid against N-nitrosodiethylamine-induced renal carcinogenesis in mice. Indian J Biochem Biophys. 2013;50:521–528.
37. Prakash M, Upadhya S, Prabhu R. Protein thiol oxidation and lipid peroxidation in patients with uraemia. Scand J Clin Lab Invest. 2004;64:599–604.
38. Peters WH, van Schaik A, Peters JH, van Goor H. Oxidised- and total non-protein bound glutathione and related thiols in gallbladder bile of patients with various gastrointestinal disorders. BMC Gastroenterol. 2007;7:7.
39. Gayathri R, Priya KDPD, Gunassekaran GR, Sakthisekaran D. Protective role of ursolic acid in den induced oxidative stress mediated hepatocellular carcinoma a focus on thiol status. Int J Pharm Pharmaceut Sci. 2010;140–146.
40. Fiala S, Mohindru A, Kettering WG, Fiala AE, Morris HP. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. J Natl Cancer Inst. 1976;57:591–598.
41. Gad AS, Khadrawy YA, El-Nekeety AA, Mohamed SR, Hassan NS, Abdel-Wahhab MA. Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. Nutrition. 2011;27:582–589.
42. Fiala S, Mohindru A, Kettering WG, Fiala AE, Morris HP. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. J Natl Cancer Inst. 1976;57:591–598.
43. Fita I, Rossmann MG. The active center of catalase. J Mol Biol. 1985;185:21–37.
44. Ozdemirler Erata G, Kanbağli O, Durlanik O, Bulut T, Toker G, Uysal M. Induced oxidative stress and decreased expression of inducible heat shock protein 70 (ihsp 70) in patients with colorectal adenocarcinomas. Jpn J Clin Oncol. 2005;35:74–78.
45. Gopčević KR, Rovčanin BR, Tatić SB, Krivokapić ZV, Gajić MM, Dragutinović VV. Activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in different stages of colorectal carcinoma. Dig Dis Sci. 2013;58:2646–2652.
46. Dai ZJ, Wu WY, Kang HF, Ma XB, Zhang SQ, Min WL, et al. Protective effects of Scutellaria barbata against rat liver tumorigenesis. Asian Pac J Cancer Prev. 2013;14:261–265.