A promising chemosesitization role of inulin in management of experimentally induced hepatocellular carcinoma

Nabil M. Abdel-Hamid,[a*] Hanaa A. Hassan,[b,c**] Hanaa M. Serag,[b] and Mahmoud M. Amr[b]

[a]Biochemistry Department, Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh, Egypt.
[b]Department Zoology, Physiology Division, Faculty of Science, Mansoura University, Mansoura, Egypt.
[c]Faculty of Science and Arts, Taibah University, Medina 42353, Saudi Arabia.
Correspondence to Nabil M. Abdel-Hamid (email: nabilmohie@yahoo.com).
(*Submitted: 10 August 2017 – Revised version received: 28 August 2017 – Accepted: 08 October 2017 – Published online: 22 December 2017*)

Objectives Globally, hepatocellular carcinoma (HCC) is an important reason of death due to cancer. The present study investigates antioxidant and anti-inflammatory properties of inulin (IN) in HCC rat model.
Methods Five groups of rats were treated for 4 months, a control group, received intra peritoneal saline daily, HCC group given freshly prepared thioacetamide (TAA) solution orally, twice weekly (200 mg/kg bw), cisplatin (CP) + TAA group (single intra peritoneal(IP) dose of CP, 7.5 mg/kg bw + TAA as mentioned), fourth group received oral doses of IN, 10 mg/kg bw, daily+TAA), the fifth group was given a single dose of CP + IN + TAA for 4 months.
Results HCC exhibited periportal fibrosis, nuclear/cytoplasmic ratio increments, significant increase in alpha fetoprotein (AFP), malondialdehyde (MDA), protein carbonyl (PC), xanthine oxidase (XO) and aldehyde oxidase (AO) activity. Hepatic glutathione (GSH), total antioxidant capacity (TAC), super oxide dismutase (SOD) and catalase (CAT) activities, were decreased. Significant decrease in B-cell lymphoma 2 (Bcl_2), while a significant increase in levels of tumor necrosis factor (TNF) and P53 in HCC group.
Conclusions Treatment of HCC rats with CP or IN improved such effects. IN was more effective than CP, and the best effect was observed using both drugs. Conclusively, IN is a chemo-sensitizer to CP in the treatment of HCC through restoration of the antioxidant defense mechanism, anti-apoptotic and anti-inflammatory properties.
Keywords Chicory seed extract, hepatocellular carcinoma, inulin, oxidative stress and inflammation

Introduction

Hepatocellular carcinoma (HCC) is the most known primary liver cancer. It represents the third cause of cancer deaths all over the world, and is the principal cause of death in patients with liver cirrhosis.[1] The known risk factors of HCC possess hepatitis C and B, alcoholic steatohepatitis, aflatoxins, cirrhosis and some metabolic diseases.[2] Although, among 25% of HCC cases still unrecognized,[3] chronic active hepatitis is the common risk for HCC, associated with hepatocyte inflammation, cytokine deregulation and cirrhosis.[4] Liver cirrhosis usually develops within a period of 20–40 years after persistent hepatic disease. TAA is a well-established inducer of liver cirrhosis.[5] The active metabolites of TAA, as TAA-sulfoxide and TAA-sulfdioxide, are hepatotoxic intermediates.[6] Administration of TAA for long time causes hyperplastic hepatic nodules, hepatocyte adenomas and HCC.[7] HCC risk exponentially increases during the cirrhosis stage, then, carcinogenic transformation takes place.[8] Cisplatin (CP) is a key drug in standard regimens in treatment of many cancers, but its clinical use is narrowed due to its side effects as nephrotoxicity,[9] neurotoxicity,[10] ototoxicity[11] and hepatotoxicity.[12] Furthermore, increasing the dose of chemotherapy to overcome resistant cancer stem cells can result in severe side effects for the patient including bone loss, hair loss and mucositis.[13] Presentation of new chemotherapeutics or enhancing the effectiveness of current chemotherapeutics could prove vital cancer care.[14] Many phytoceutical antioxidants showed favourable effects on human diseases.[15] Inulin (IN) is obtained from chicory roots (Cichorium intybus L.), as a mixture of linear polymers and oligomers composed of fructose joined through β(2–1) glycosidic linkage mostly by glucose ending.[16] It shows antioxidant activity, preventing liver cell damage, through capturing oxidants, maintaining the native mechanisms that protect the cell.[17] Thus, it was thought worthwhile to investigate the ability of supplementation of IN as a possible sensitizer for CP treatment and evaluate its antioxidant, anti-apoptotic and anti-inflammatory properties in thioacetamide (TAA)-induced HCC model.

Materials and Methods

Experimental animals

Two months old adult male Wistar rats (120–150 g), have been purchased from the Institute of Ophthalmic Disease Research in Cairo (Egypt). They were kept in stainless cages in a clean room with adjusted temperature (22–25°C) and standard chow and water ad libitum. Light cycle as equal light and darkness periods was maintained.

Chemicals and drugs

TAA and IN were purchased from Sigma Chemical Co., St. Louis, MO, USA. CP was purchased from local Egyptian market (Mansoura, Egypt). TAA was used to induce chronic liver damage and hepatocarcinoma.[18]

Experimental design

Rats were classified into five groups (six rats per each): group I, rats served as control received intra peritoneal (IP) saline daily, group II rats, injected by fresh TTA solution twice weekly at a dose of 200 mg/kg b.wt (IP)[19], for 4 months, group III rats given CP (7.5 mg/kg/bw) as a single IP dose[20] in addition to TAA, as

mentioned in group II. Group IV rats were orally administered IN (10 mg /kg/bw)[21] in addition to TAA with for 4 months and group V rats were given CP (7.5 mg/kg bw) and IN in addition to TAA (200 mg/kg bw), as before, for 4 months.

Collection of samples

By the last day of work, all animals were kept fasting to the morning, venous blood samples were withdrawn, centrifuged at 860×g for 20 min at 4°C and kept at –20°C right analysis. Rats were dissected and the liver tissue was divided into 2 parts, one was kept in formal saline for microscopic investigation and the other was weighed, minced in saline with Potter-Elvehjem type homogenizer on an ice container. Homogenate was centrifuged at 860×g for 20 min at 40°C, and supernatants were frozen at –20°C right analysis.

Biochemical analysis

These investigations comprised two parts, one in the serum as: Alpha fetoprotein (AFP), TAC, tumor necrosis factor (TNF)-α, P53 and B-cell lymphoma 2 (Bcl_2), the other in liver tissue as: MDA, GHS, PC, AO, XO and CAT.

Quantitative measurement of AFP level in serum was executed by ELISA according to the method of Acosta (1983), kits were purchased from Diagnostic, Pacific Concourse, Drive, Los Angeles, USA. Malondialdehyde (MDA) and protein carbonyl (PC) contents were assessed according to the methods of Ohkawa et al.[22] and Smith et al.[23], respectively. Xanthine oxidase (XO) and aldehyde oxidase (AO) activity were determined as described previously.[24,25] Reduced glutathione (GSH) content was assayed by the method of Prins and Losse.[26] Total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT) activities were estimated as described previously,[27–29] Serum TNF-α level was determined by ELISA (Diagnostic Products Corp, Los Angeles, CA, USA) as described by Aggarawal.[30] Bcl_2 and P53 levels were computed.[31,32]

Statistical analysis

Results were recorded as mean ± SE. Statistical significance was computed exploiting one-way analysis of variance (ANOVA).[33] We used SPSS 12.00 software. Variations of *t*-value less than $P \leq 0.05$, were considered significant.

Results

Histological examination showed normal tissue architecture of control rats, while HCC group showed fibrosis as blue collagen fibers around CV with few nuclear/cytoplasmic ratio increments. Liver sections of group 2 (HCC + Cis) showed little strands of blue collagen in hepatocytes. HCC + IN liver sections showed similar features to control rats group. HCC + Cis + IN showed approximately normal hepatocytes (Fig. 1).

As shown in Table 1, a significant increase in oxidative stress biomarkers (MDA and PC) as well as free radical enzymes activity (AO and XO accompanied with significant decrease in enzymatic and non-enzymatic antioxidants (GSH, TAC, SOD and CAT), in HCC rats comparing to control. In contrast, administration of IN or CP to HCC rats showed amelioration in the tested parameters. Moreover, the results in Table 2, showed a significant increase in the level of serum AFP, TNF-α and P53 but decrease in BCl_2 level in HCC rats than control. However, administration of IN or CP

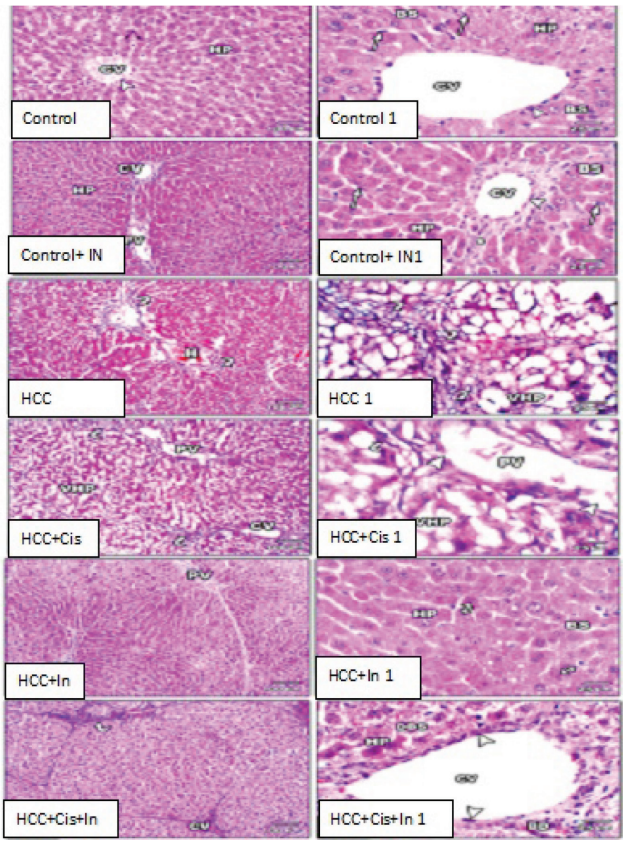

Figure 1 **Histopathological liver changes, in response to thioacetamide, cisplatin and inulin administration after 4 months of treatment (H&E):**

to HCC rats succeeded to attenuate these changes. Furthermore, the IN is more effective than CP and could sensitize cellular response to CP.

Discussion

HCC seems to be the most lethal of all cancers. A lot of dietary,[34] endogenous and environmental,[35] stimuli mediate hepatocarcinogenesis. Carcinogenesis may result from biological or chemical effects on normal cells through multistage and complicated steps, involving changes of the initiation step, promotion, progression that ends by malignant transformation.[36] Chemical carcinogens initiate oxidative stress on different cellular compartments, ultimately complicate to malignancy.[37]

TAA was adopted as a well trusted a chemical that can induce liver cirrhosis in rats, with a very close human cirrhotic analogy.[4] Intra peritoneal TAA generates fibrosis, cirrhosis and HCC in rats.[38] It is a sulfur(thio) containing necrogenic[39] and carcinogenic[40] potentials. Experimentally, it is acceptable inducer of fulminant hepatic failure and liver cirrhosis in animals.[41] Biotransformation of TAA into oxidative products is responsible for hepatic damage, so that, flavin-containing monooxygenase (FMO)[42] and cytochrome P450[43] reduces dioxygen into superoxide anion, that is metabolized into H_2O_2.[44]

HCC disturbs liver functions through oxidative cellular derangements induced by reactive oxygen species (ROS) causing lipid peroxidation, a risk for damage to the macromolecules in most of bio membranes.[45] In our study, administration of a single dose of TAA could induce premalignant

Table 1. **Effect of inulin co administration with/without cisplatin on oxidative stress profile in thioacetamide induced liver injury, in rats. Values are expressed as mean ± SE (N=6)**

Parameters	Animal groups				
	C	HCC (TAA)	TAA+CP	TAA+ In	TAA + In + CP
MDA (n mol/g)	33.4±0.3[a]	76.80±2.91[b]	59.45±1.40[c]	54.40±1.40[d]	49.84±1.16[e]
PC (µmol DNPH/g)	0.33±0.01[a]	0.82±0.01[b]	0.45±0.02[c]	0.41±0.02[d]	0.39±0.02[e]
AO (µmole/h/g)	0.46±0.02[a]	1.73±0.15[b]	0.86±0.03[c]	0.77±0.05[d]	0.55±0.02[a]
XO (µmole/h/g)	0.26±0.01[a]	0.92±0.05[b]	0.65±0.03[c]	0.59±0.02[d]	0.52±0.01[d]
GSH (mg/g)	0.46±0.004[a]	0.27±0.006[b]	0.38±0.02[c]	0.40±0.006[d]	0.45±0.009[a]
TAC (mM/L)	0.86±0.03[a]	0.23±0.01[b]	0.38±0.01[c]	0.47±0.01[d]	0.58±0.02[e]
SOD (U/g)	192.99±1.18[a]	140.56±2.49[b]	180.09±1.98[c]	183.99±1.35[d]	188.85±1.16[e]
CAT(µmol/sec/g)	10.13±0.19[a]	4.55±0.38[b]	5.44±0.35[c]	6.68±0.35[b]	7.99±0.22[c]

Values superscripted with different letters (a-e) were significantly different (P≤ 0.05), but with the same letters were non significantly different.

Table 2. **Effect of inulin co administration with/without cisplatin on serum AFP and apoptotic profile including tumor necrosis factor (TNF-α) in thioacetamide induced liver injury, in rats. Values are expressed as mean ± SE (N=6)**

	Animal groups				
Parameters	C	HCC (TAA)	TAA+CP	TAA+ In	TAA + In + CP
AFP(pg/ml)	26.7±1.3	59.5±3.2	42.1±1.1	36.6±1	31.8±0.7
	109.6±7.3[a]	249.3±1.47[b]	179.9±1.2[c]	145.44±1.2[d]	134.3±2.2[e]
P53 (Pg/ml)	25.7±1.2[a]	60.78±1.8[b]	43.50±1.02[c]	37±0.76[d]	31.4±0.52[e]
BCl2 (Pg/ml)	61.7±0.6[a]	23.5±1.4[b]	32.3±0.07[c]	37±0.6[d]	50.1±1.3[e]

Values superscripted with different letters (a-e) were significantly different (P≤ 0.05), but with the same letters were non significantly different.

hepatic injury, which has been proven by the significant tissue changes, as well as, elevated AFP levels, alterations in oxidative stress markers (MDA and PC), depressed antioxidant states that may be referred to an increase of ROS by TAA intoxication.[46] The final compounds of lipid peroxidation yield a variety of highly reactive electrophilic aldehydes, which can act as endogenous danger signals that alter important cell signaling pathways responsible for disease pathogenesis such as inflammation, atherosclerosis, diabetes, ageing, neurodegenerative ailments, and malignancy.[47]

It was frequently observed that oxidative stress contributed in carcinogenesis.[48] XO and AO are molybdo-flavoenzyme containing hydroxylases, known to contribute in various forms of injury.[49] Elevated activity of these enzymes produce ROS and therefore lead to oxidative stress.[50–53]

These enzymes contribute to the catabolism of purines in humans.[54] In the present project, superoxide dismutase (SOD) activity was reduced, TAC and CAT activities, and reduced GSH content. The downregulated SOD activity assumes deactivation of this enzyme, presumably due to elevated superoxide release or depression by H_2O_2 due to decreased CAT activity, that preferentially breaks H_2O_2.[55] The depressed GSH, SOD and CAT in HCC rats can be attributed to elevation of ROS. SOD is deemed as to be the confront of defense against harmful effects of intracellular ROS through catalysis by superoxide radicals (O_2^-) to H_2O_2 and O_2. CAT detoxifies H_2O_2, which in turn is potent inhibitor of SOD.[56] Kregel and Zhang[57] attributed the downregulation in SOD and CAT activities to elevated ROS, which interferes with the enzyme through denaturation and inactivation. The current study indicated that HCC causes increase in inflammatory chemokines as TNF-α [58] and apoptotic cell marker (p53) accompanied with decrease anti-apoptotic proteins (Bcl-2). These findings were reported in cirrhosis, fibrosis and cancer.[59]

HCC was reported to be associated by failure in the anti-apoptotic protein Bcl_2 maintenance and its translocation into the mitochondria, this hinders apoptosis.[60] Further studies published that the ROS induced by TAA metabolites induce necrosis.[61] In our work, the significant downregulation of blood level of Bcl-2 protein interprets the liability of cells to apoptosis by TAA actions.[62] A dilemma of mechanisms are sought in CP resistance, as depressed intracellular accumulation, drug deactivation by elevated contents of cellular thiols, changes in drug target, metabolism or drug-induced damage by elevated nucleotide excision-repair activity and decreased mismatch-repair activity and evasion of apoptosis.[63]

On the other hand, it has been established that chicory-supplemented diet improved antioxidant activities against ROS responsible for cellular damage and elevated liver functions.[14] Previous records reported that chicory significantly reduces the oxidative stress, restoring GSH and CAT levels, thereby up-regulates the native antioxidant defense networking.[64] In this study, administration of IN sensitized the used chemotherapy through a decrease in oxidative stress variables and an increase in

antioxidant defense with decreased apoptotic cell marker (p53) and inflammatory cytokines (TNF-α) accompanied with increased anti apoptotic cell marker (BCl_2). IN is scavenger to ROS possibly due to the NH_2 grafted in its molecule. NH_2 can be converted to NH_3 by absorbing hydrogen ion from media, then react with ·OH[65]. IN is extracted from chicory, bacterial fermentation of chicory fructans yields short-chain fatty acids in colon, as butyric acid.[16] It activates the immune system, prevents inflammation and bacterial infections.[64]

Conclusion

Our data indicated the efficacy of IN as a chemo-sensitizer for CP treatment in experimentally induced HCC. This hypothesis is supported by up-regulation of the native antioxidant system and capturing ROS responsible for oxidative stress and cell damage. It also elevates the apoptotic potential of hepatocytes, through modulation of TNF-α, p53 and Bcl_2, which is manifested by decreased serum AFP and normalized liver tissue.

Author Contributions

Nabil contributed in conception, design and critical review, Hanaa Hassan and Hanaa Serag, in conception , analysis and drafting. Mahmoud Amr contributed to analysis and drafting of the manuscript.

Declaration of Conflicting Interests

The authors declare no conflict of interest with respect to the manuscript, authorship, and/or publication of the manuscript.

Funding

The authors declare that the research didn't receive funding from anybody.

Ethical Approval

All procedures performed in this study comply with ethical guidelines of the University Research Committee. ■

References

1. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132:2557–2576.
2. Schutte K, Bornschein J, Malfertheiner P. Hepatocellular carcinoma—epidemiological trends and risk factors. Dig Dis. 2009;27:80–92.
3. Parikh S, Hyman D. Hepatocellular cancer: a guide for the internist. Am J Med. 2007;120:194–202.
4. Lei HY, Chang CP. Lectin of Concanavalin A as an anti-hepatoma therapeutic agent. J Biomed Sci. 2009;16:10.
5. Aydin AF, Küskü-Kiraz Z, Doğru-Abbasoğlu S, Güllüoğlu M, Uysal M, Koçak-Toker N. Effect of carnosine against thioacetamide-induced liver cirrhosis in rat. Peptides. 2010;31:67–71.
6. Novosyadlyy R, Dargel R, Scharf JG. Expression of insulin-like growth factor-I and insulin-like growth factor binding proteins during thioacetamide-induced liver cirrhosis in rats. Growth Horm IGF Res. 2005;15:313–323.
7. Yeh CN, Maitra A, Lee KF, Jan YY, Chen MF. Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: an animal model recapitulating the multi-stage progression of human cholangiocarcinoma. Carcinogenesis. 2004; 25:631–636.
8. Yang MC, Chang CP, Lei HY. Induction of liver fibrosis in a murine hepatoma model by thioacetamide is associated with enhanced tumor growth and suppressed antitumor immunity. Lab Invest. 2010;90:1782–1793.
9. Hassan HA, Edrees GM, El-Gamel EM, El-Sayed EA. Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage. Cytotechnology. 2014;66:419–429.
10. Barabas K, Milner R, Lurie D, Adin C. Cisplatin: a review of toxicities and therapeutic applications. Vet Comp Oncol. 2008;6:1–18.
11. Rybak LP, Mukherjea D, Jajoo S, Ramkumar V. Cisplatin ototoxicity and protection: clinical and experimental studies. Tohoku J Exp Med. 2009; 219:177–186.
12. Liao Y, Lu X, Lu C, Li G, Jin Y, Tang H. Selection of agents for prevention of cisplatin-induced hepatotoxicity. Pharmacol Res. 2008;57:125–131.
13. Cortes J, Radich J, Mauro MJ. Clinical roundtable monograph: Emerging treatment options for TKI-resistant chronic myelogenous leukemia. Clin Advan Hematol Oncol. 2012;10:1–16.
14. Schoener CA, Carillo-Conde B, Hutson HN, Peppas NA. An inulin and doxorubicin conjugate for improving cancer therapy. J Drug Deliv Sci Technol. 2013;23:111–118.
15. Hassan HA, El-Gharib NE. Obesity and clinical riskiness relationship: therapeutic management by dietary antioxidant supplementation—a review. Appl Biochem Biotechnol. 2015;176:647–669.
16. Roberfroid MB, Van Loo JA, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. J Nutr. 1998;128:11–19.
17. Saggu S, Sakeran MI, Zidan N, Tousson E, Mohan A, Rehman H. Ameliorating effect of chicory (Chichorium intybus L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. Food Chem Toxicol. 2014;72:138–146.
18. Muñoz Torres E, Paz Bouza JI, López Bravo A, Abad Hernández MM, Carrascal Marino E. Experimental thioacetamide-induced cirrhosis of the liver. Histol Histopathol. 1991;6:95–100.
19. Toyama T, Nakamura H, Harano Y, Yamauchi N, Morita A, Kirishima T, et al. PPARalpha ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. Biochem Biophys Res Commun. 2004;324:697–704.
20. Yilmaz HR, Iraz M, Sogut S, Ozyurt H, Yildirim Z, Akyol O, et al. The effects of erdosteine on the activities of some metabolic enzymes during cisplatin-induced nephrotoxicity in rats. Pharmacol Res. 2004;50:287–290.
21. Rault-Nania MH, Demougeot C, Gueux E, Berthelot A, Dzimira S, Rayssiguier Y, et al. Inulin supplementation prevents high fructose diet-induced hypertension in rats. Clin Nutr. 2008;27:276–282.
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–358.
23. Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, et al. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA. 1991; 88: 10540–10543.
24. Stirpe F, Della Corte E. The regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J Biol Chem. 1969;244:3855–3863.
25. Johnson C, Stubley-Beedham C, Stell JG. Elevation of molybdenum hydroxylase levels in rabbit liver after ingestion of phthalazine or its hydroxylated metabolite. Biochem Pharmacol. 1984;33:3699–3705.
26. Prins HK, Loose JA. Biochemical Methods in Red Cell Genetics. (J.J. Unis ed.) Academic Press, New York. 1969. p. 115.
27. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001; 54:356–361.
28. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972;46:849–854.
29. Bock PP, Karmer R, Paverka M. A simple assay for catalase determination. Cell Biol Monogr. 1980;7:44–74.
30. Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ, et al. Human tumor necrosis factor. Production, purification, and characterization. J Biol Chem. 1985;260:2345–2354.
31. Aiello A, Delia D, Borrello MG, Biassoni D, Giardini R, Fontanella E, et al. Flow cytometric detection of the mitochondrial BCL-2 protein in normal and neoplastic human lymphoid cells. Cytometry. 1992;13:502–509.
32. Brotherick I, Shenton BK, Cowan WK, Angus B, Horne CH, Higgs MJ, et al. p53 expression measured by flow cytometry. A comparison of three monoclonal antibodies and the relationship with grade and DNA ploidy in breast cancer. Cancer Immunol Immunother. 1995;41:146–150.
33. Waller RA, Duncan DB. Bayes rule for the symmetric multiple comparison problems. J Am Stat Assoc. 1969;64:1484–1503.

34. Chen B, Ning M, Yang G. Effect of paeonol on antioxidant and immune regulatory activity in hepatocellular carcinoma rats. Molecules. 2012; 17:4672–4683.
35. Guyton KZ, Kensler TW. Oxidative mechanisms in carcinogenesis. Br Med Bull. 1993;49:523–544.
36. Kalaiselvan A, Anand T, Gokulakrishnan K, Kamaraj MC, Velavan S. Modulatory role of Shorea robusta bark on glucose-metabolizing enzymes in diethylnitrosamine induced hepatocellular carcinoma in rats. Pharmacog Magazine. 2015;11:S496–S500.
37. Trush MA, Kensler TW. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Rad Biol Med. 1991;10:201–209.
38. Zhao G, Nakano K, Chijiiwa K, Ueda J, Tanaka M. Inhibited activities in CCAAT/enhancer-binding protein, activating protein-1 and cyclins after hepatectomy in rats with thioacetamide-induced liver cirrhosis. Biochem Biophys Res Commun. 2002;292:474–481.
39. Landon EJ, Naukam RJ, Rama Sastry BV. Effects of calcium channel blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents. Biochem Pharmacol. 1986;35:697–705.
40. Kizer DE, Clouse JA, Ringer DP, Hanson-Painton O, Vaz AD, Palakodety RB, et al. Assessment of rat liver microsomal epoxide hydrolase as a marker of hepatocarcinogenesis. Biochem Pharmacol. 1985; 34:1795–1800.
41. Li X, Benjamin IS, Alexander B. Reproducible production of thioacetamide-induced macronodular cirrhosis in the rat with no mortality. J Hepatol. 2002; 36:488–493.
42. Malvaldi G, Chieli E, Saviozzi M. Biliary cirrhosis and tumors induced by chronic administration of thiobenzamide to rats. Arch Toxicol. 1984;55:34–38.
43. Lee AJ, Conney AH, Zhu BT. Human cytochrome P450 3A7 has a distinct high catalytic activity for the 16alpha-hydroxylation of estrone but not 17beta-estradiol. Cancer Res. 2003;63:6532–6536.
44. Ekström G, von Bahr C, Ingelman-Sundberg M. Human liver microsomal cytochrome P-450IIE1. Immunological evaluation of its contribution to microsomal ethanol oxidation, carbon tetrachloride reduction and NADPH oxidase activity. Biochem Pharmacol. 1989;38:689–693.
45. Mohamed AM, Metwally NS, Mohamed SM, Hassan EM. Protective capacity of butanolic extract of myoporum laetum against oxidative stress and immune disorder induced tissue damage in profenofos intoxicated rats . J Int Acad Res. 2010;2:157–164.
46. Ansil PN, Nitha A, Prabha SP, Wills PJ, Jazaira V, Latha MS. Protective effect of Amorphophallus campanulatus (Roxb.) Blume. tuber against thioacetamide induced oxidative stress in rats. Asian Pac J Trop Med. 2011;4:870–877.
47. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438.
48. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol. 2010;38:96–109.
49. Ali S, Pawa S, Naime M, Prasad R, Ahmad T, Farooqui H, et al. Role of mammalian cytosolic molybdenum Fe-S flavin hydroxylases in hepatic injury. Life Sciences. 2008;82:780–788.
50. Zargar S. Protective effect of Trigonella foenum-graecum on thioacetamide induced hepatotoxicity in rats. Saudi J Biol Sci. 2014;21:139–145.
51. Sunitha S, Nagaraj M, Varalakshmi P. Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats. Fitoterapia. 2001;72:516–523.
52. Chilakapati J, Korrapati MC, Hill RA, Warbritton A, Latendresse JR, Mehendale HM. Toxicokinetics and toxicity of thioacetamide sulfoxide: a metabolite of thioacetamide. Toxicology. 2007;230:105–116.
53. Balkan J, Doğru-Abbasoğlu S, Kanbağli O, Cevikbaş U, Aykaç-Toker G, Uysal M. Taurine has a protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress. Hum Exp Toxicol. 2001;20: 251–254.
54. Hille R. Molybdenum-containing hydroxylases. Arch Biochem Biophys. 2005; 433:107–116.
55. El Shahat AN. Efficiency of broccoli in attenuating of some biochemical disorders in rats exposed to γ-irradiation. Arab J Nuc Sci Applic. 2013;46:260–267.
56. de Duve C. The peroxisome in retrospect. Ann N Y Acad Sci. 1996;804:1–10.
57. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regulat Integrat Compar Physiol. 2007;292:R18–R36.
58. Weber A, Boege Y, Reisinger F, Heikenwälder M. Chronic liver inflammation and hepatocellular carcinoma: persistence matters. Swiss Med Wkly. 2011;141:w13197.
59. Lai PB, Chi TY, Chen GG. Different levels of p53 induced either apoptosis or cell cycle arrest in a doxycycline-regulated hepatocellular carcinoma cell line in vitro. Apoptosis. 2007;12:387–393.
60. Chen LH, Hsu CY, Weng CF. Involvement of P53 and Bax/Bad triggering apoptosis in thioacetamide-induced hepatic epithelial cells. World J Gastroenterol. 2006;12:5175–5181.
61. Sarkar MK, Sil PC. Hepatocytes are protected by herb *Phyllanthus niruri* protein isolate against thioacetamide toxicity. Pathophysiology ISP. 2007;14 :113–120.
62. Wang ME, Chen YC, Chen IS, Hsieh SC, Chen SS, Chiu CH. Curcumin protects against thioacetamide-induced hepatic fibrosis by attenuating the inflammatory response and inducing apoptosis of damaged hepatocytes. J Nutr Biochem. 2012;23:1352–1366.
63. Brabec V, Kasparkova J. Modifications of DNA by platinum complexes. Relation to resistance of tumors to platinum antitumor drugs. Drug Resist Updat. 2005;8:131–146.
64. Ghamarian A, Abdollahi M, Su X, Amiri A, Ahadi A, Nowrouzi A. Effect of chicory seed extract on glucose tolerance test (GTT) and metabolic profile in early and late stage diabetic rats. Daru J Faculty Pharm. 2012;20:56.
65. Xie W, Xu P, Liu Q. Antioxidant activity of water-soluble chitosan derivatives. Bioorg Med Chem Lett. 2001;11:1699–1701.

This work is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported License which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.