Alterations in Lipid Peroxidation and Antioxidants in Patients' with Different Stages of Hepatitis B Virus Infection in Egypt

Nadia Z. Shaban*[1], Halima H. Salem[2], Mohamed A. Elsadany[1], Bahy A. Ali[2], Ehab M Hassona[3] and Fayed A. K. Mogahed[2]

[1]Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt
[2] Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt
[3] Internal Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
nshaban2001@yahoo.co.uk

Abstract: Persistent hepatitis B virus (HBV) infection often leads to the development of chronic hepatitis, cirrhosis and hepatocellular carcinoma. The aim of this study was to estimate the oxidant/antioxidant status and liver function tests in different stages of HBV infection to investigate the role of oxidative stress in the pathogenesis of chronic liver diseases. The study was carried out on 150 control healthy persons (C) and 320 patients with HBV infection which included normal carriers (N), acute (A), chronic (CH), cirrhosis (Cl) and hepatocellular carcinoma (HCC). The study focused on the determination of malondialdehyde (MDA), non enzymatic and enzymatic antioxidant parameters and liver function profile tests. The results showed that MDA and glutathione reductase (GSR) were increased, while reduced glutathione (GSH), total glutathione s-transferase (GST), total glutathione peroxidase (GPx), superoxide dismutase (SOD) were decreased in N, A, CH, CI and HCC groups as compared to the C group. Also, the levels of total protein (TP) and albumin were decreased but alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and total bilirubin (TB) level were increased in all studied groups as compared to the C group. This indicates that HBV induced oxidative stress, lipid peroxidation LPO, liver injury and cell death. We concluded that the determination of antioxidant system, oxidative stress and liver function tests may be considered as accurate biomarkers for determining the progression of HBV infection. Also treatment with antioxidant supplementation is very important in case of HBV infection.
[Nadia Z. Shaban, Halima H. Salem, Mohamed A. Elsadany , Bahy A. Ali, Ehab M Hassona and Fayed A. K. Mogahed. **Alterations in Lipid Peroxidation and antioxidants in Patients' with different stages of Hepatitis B Virus infection in Egypt.** *Life Sci J* 2014;11(8):960-967]. (ISSN:1097-8135). http://www.lifesciencesite.com. 141

Key Words: Hepatitis B virus, Liver function tests, Oxidative stress, Antioxidant enzymes.

1. Introduction

Oxidation is a metabolic process that leads to energy production necessary for essential cell activities. However, metabolism of oxygen in living cells also leads to the unavoidable production of reactive oxygen species (ROS) such as superoxide ($O_2^{-\cdot}$) radical, hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). The $O_2^{-\cdot}$ radical can react with nitric oxide to form the peroxynitrite radical ($ONOO^{\cdot}$) (1). There are two sources of free radicals, endogenous and exogenous sources. The endogenous FRs is derived from normal cellular metabolism and oxidative burst produced when phagocytic cells destroy invading microorganisms such as bacteria and viruses. Also the reactions of certain oxidases, cyclooxygenases, lipoxygenases, dehydrogenases, and peroxidases are the sources of ROS (1, 2). The exogenous sources of free radicals include tobacco smoke, air pollutants, organic solvents, anesthetics, pesticides, drugs, and radiation (3, 1). ROS have enormous ability to cause damage to biomolecules (lipids, protein or DNA), cells and tissues because of their highly reactive nature (4). ROS alterations in different signaling pathways may modulate gene expression, cell adhesion, cell metabolism, cell cycle and cell death (5). It has been reported that ROS are potential carcinogens because of their roles in mutagenesis, tumor promotion, and progression **(**5**)**.

Polyunsaturated fatty acids (PUFAs) residues of phospholipids located in cell membranes and intracellular organelles are highly susceptible to reaction with free radicals. In addition, fatty acids that esterified in glycolipids, or cholesterol esters can undergo lipid peroxidation (LPO) by the help of free radicals (6). The LPO products such as aldehydes (malondialdehyde and hydroxynonenal), are capable of forming crosslinks with lipids, proteins, and nucleic acids, thereby causing damage to the macromolecules, which are essential components of biological tissues (1, 7).

A number of sophisticated antioxidant systems exist in aerobic organisms, and their unction interactively and synergistically to balance the cellular production of ROS. They involve a variety of components, both endogenous and exogenous in origin (8). Endogenous antioxidant defenses include a

network of compartmentalized antioxidant enzymes that are usually distributed within the cytoplasm and among various organelles in cells. A variety of small non-enzymatic molecules present in the internal milieu are also capable of scavenging ROS. In eukaryotic organisms, several ubiquitous primary antioxidant enzymes are superoxide dismutase (SOD), catalase, and different forms of peroxidases. They work in a complex series of integrated reactions to convert ROS to more stable molecules such as water and O_2 as shown in Fig. 1 (9). These enzymes require micronutrients as cofactors such as selenium, iron, copper, zinc, and manganese for optimal catalytic activity and effective antioxidative defense mechanism. Besides the primary antioxidant enzymes, a large number of secondary enzymes present such as glutathione reductase, glucose-6- phosphate dehydrogenase. These enzymes help in maintaining a steady concentration of glutathione and NADPH necessary for optimal functioning of the primary antioxidant enzymes (10). Detoxicant enzymes, such as glutathione *S*-transferase (GST), metabolize toxic electrophiles and are considered also to be secondary antioxidant enzyme (11). Oxidative stress (OS) is a disturbance in the oxidant-antioxidant balance leading to potential cellular damage. Most cells can tolerate a mild degree of OS. The imbalance can result from a lack of antioxidant capacity caused by disturbances in production and distribution, or by an overabundance of ROS from other factors (3).

Extensive liver injury of various etiology is manifested as hepatocyte damage and associated with inflammatory and immune responses. It is the starting point for events which may result in fibrosis, often followed by cirrhosis (12). Liver damage commonly results by viral and protozoal infection, toxicity due to drugs, food additives and fungal toxins (13).

Hepatitis B is one of the world's most serious and widespread chronic diseases (14). The World Health Organization (WHO) estimates that hepatitis B infection leads to more than one million deaths every year (15). HBV infection can result in acute hepatitis, HBV carriage, chronic hepatitis, liver cirrhosis, and even primary hepatocellular carcinoma (HCC). Progression of hepatitis to cirrhosis and the response to therapy are variable among individuals. This variability is due to the primary cause of disease and inter-individual differences in target proteins and drug metabolism (16). Also the progress of HBV infection may be affected by host genetic susceptibility (17). It has been found that the total peroxide level is significantly higher in patients with CH. Also lipid peroxidation and oxidative DNA damage are enhanced in patients with HBV infection (18). In addition the previous studies showed that ROS play a pathogenetic role in carcinogenesis (19). Therefore this study was carried out to investigate the changes in lipid peroxidation and the activities of the antioxidant enzymes in human liver at different stages of HBV. Also the study included the determination of liver function profile.

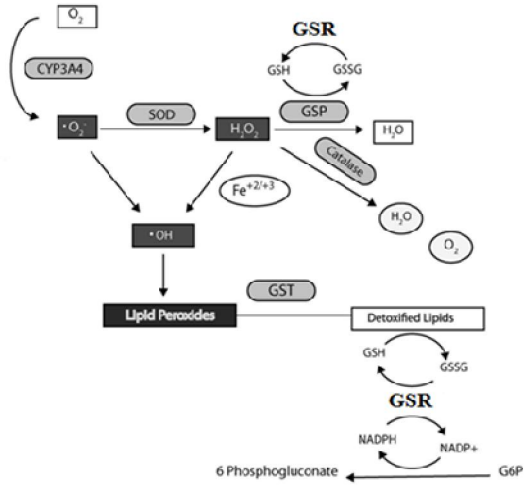

Fig. 1: Chemical equations of antioxidant enzymes. Since SOD = superoxide dismutase, CAT = catalase, GPx, = glutathione peroxidase, GSR = glutathione reductase, GST = glutathione S transferase, CYP3A4 = cytochrome P 3A4 and G6P = glucose 6 phosphate.

2-Materials and Methods

2.1. Chemicals and kits:

GST assay kit, OxiSelecTM MDA Quantitative assay kit, EnzyChromTM GSH/GSSG assay, SOD assay and GSR assay kits were obtained from Cornel lab, Cell Biolab, BioAssay Systems, Cyman, USA, respectively. Total GPX Assay kit was obtained from Gentaur, Belgium. Total protein kit was obtained from Spinreact, Spain. Albumin kit was obtained from Borax diagnostic, England. AST kit was obtained from Prodia international, Germany. ALT kit was obtained from Crest biosystem, India and Bilirubin kit was obtained from Diamond, Egypt.

2.2. Patients and Control:

The present study was carried out on blood samples of 470 persons (310 males and 160 females) which included 150 healthy persons (control group, C) and 320 patients with HBV: The age of studied cases was (43±13) years. The patients were classified into five groups according to clinical history, serological tests and histological analysis as follow: 67 normal carriers (N), 53 acute hepatitis (A), 78 chronic hepatitis (CH), 62 cirrhotic liver (CL), and 60 hepatocellular carcinoma (HCC). Complete demographic date and full clinical history were recorded. Different blood samples were collected from different hospitals, medical laboratories and

hepatologist clinics in Egypt according to the rules of scientific research ethics.

Serum preparation: Five ml of blood from each person were collected, left at room temperature for 10 min and then centrifuged at 8000 rpm for 5 min at 4 ºC. All serum samples were stored at -80 ºC.

2.3. Biochemical Assays:

2.3.1. Determination of molondialdehyde (MDA): MDA, a marker of lipid peroxidation, was determined using kit according to the thiobarbituric acid (TBA) test (20). The pink product was measured spectrophotometrically at 530 nm. The specific unit is defined as µmol of MDA/L.

2.3.2. Determination of reduced glutathione (GSH): The GSH concentration in blood was determined according to the method of Hu *et al.*, (21) using kit. 2-dinitrobenzoic acid (DNTB) was reacted with GSH giving 2-nitro-5-thiobenzoic acid, a yellow colored product with a maximum absorbance at 412 nm which determined specthrophotometrically. The specific unit is defined as mg/dL.

2.3.3. Superoxide dismutase (SOD, EC 1.15.1.1) activity: The superoxide dismutase was determined using kit by the indirect method using pyrogallol as described by Michiels *et al* (22). The product was read at 420 nm and the specific unit is defined as U/mL.

2.3.4. Total Glutathione peroxidase (t-GPx, EC 1.11.1.9) activity: T-GPx activity was determined spectrophotometrically using kit in which cumene hydroperoxide acts as a substrate (23). The product was read at 366 nm and the specific unit is defined as U/L.

2.3.5. Glutathione reductase (GSR, EC 1.8.1.7) activity:

GSR is a ubiquitous NADPH-dependent enzyme which catalyzes the reduction of oxidized glutathione (GSSG) to GSH and determined spectrophotometrically (24) using kit. The product was measured at 340 nm and the specific unit is defined as U/L.

2.3.6. **Total Glutathione S-transferase, t-GST (EC** 2.5.1.18) **activity:** GST assay kit measures total GST activity by measuring the conjugation of 1-chlor 2-4 dinitrobenzene (CDNB) with reduced glutathione forming a conjugate product. This conjugation is accompanied by an increase in absorbance at 340 nm (25). The specific activity is defined as U/L.

2.3.7. Liver function tests: The transaminases [aspartate tranaminase (AST) and alanine transaminase (ALT)], total protein (TP), albumin and total bilirubin (TB) were determined using appropriate kits (26).

3. Results

3.1. MDA concentration: The results showed that serum MDA levels were increased significantly in N, A, CH, Cl and HCC groups by about 39.4 %, 52.4 %, 100.9%, 133.2 % and 168.5 %, respectively as compared to the C group (Fig. 2).

3.2. Reduced (GSH) level: The results showed that GSH levels were decreased significantly in N, A, CH, CI and HCC groups by about 38.4 %, 44.9 %, 52.8%, 65 % and 72.4 %, respectively compared to the C group (Fig. 3 a).

3.3. GSR activity: The results showed that GSR activities were increased significantly in N, A, CH, Cl and HCC groups by about 23.2 %, 40.7 %, 64.1%, 90.6 % and 187.9 %, respectively as compared to the C group as compared to the C group (Fig. 3 b).

3.4. SOD activity: SOD activities were decreased significantly in N, A, CH, Cl and HCC groups by about 45.6 %, 53.0 %, 57.7%, 64.8 % and 76.8 %, respectively as compared to the C group (Fig. 3 c).

3.5. T-GPx activity: As shown from Fig. (3 d), the activities t-GPx were decreased significantly in N, A, CH, Cl and HCC groups by about 57.1 %, 64.8 %, 75.5%, 80.0 % and 84.5 %, respectively as compared to the C group.

3.6. T-GST activity: The results showed that the GST activities were decreased significantly in N, A, CH, Cl and HCC groups by about 40.1 %, 52.9 %, 57%, 54.3 % and 65.8 %, respectively as compared to the control group (Fig. 3 e).

3.7. Liver function tests: The levels of TP and albumin were decreased significantly in N, A, CH, Cl and HCC groups as compared to the control group (Table 1). However AST and ALT activities and TB levels were increased in all groups compared to the control group (Table 1).

Table 1: Liver function tests in different stages of HBV infection

Studied groups	Total protein (Mean ±SD)	Albumin (Mean ±SD)	AST (Mean ±SD)	ALT (Mean ±SD)	Total bilirubin (Mean ±SD)
Control	7.55	4.77	11.88	13.65	0.83
Normal	4.87	2.88	25.53	27.16	4.1
Acute	3.67	2.32	32.06	53.05	5.94
Chronic	3.91	1.81	27.2	17.03	5.15
Cirrhosis	3.83	1.77	42.9	25.32	7.31
HCC	3.41	1.45	23.41	60.33	8.35
***P*. value**	0.003	0.01	0.02	0.008	0.001

Values of ***P* <0.05** were considered statistically significant.

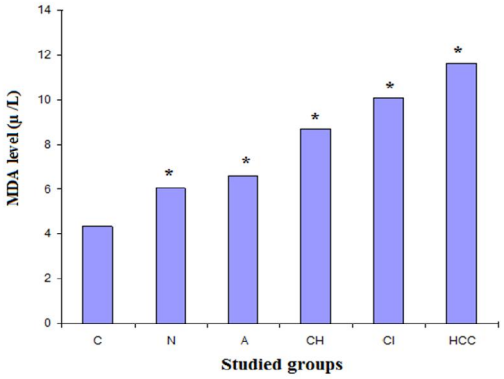

Fig. 2: Malonalaldehyde (MDA) in different stages of HBV infection.
Since N=normal carriers, A=acute infection, CH=chronic infection, Cl= cirrhotic liver and HCC=hepatocellular carcinoma patients.

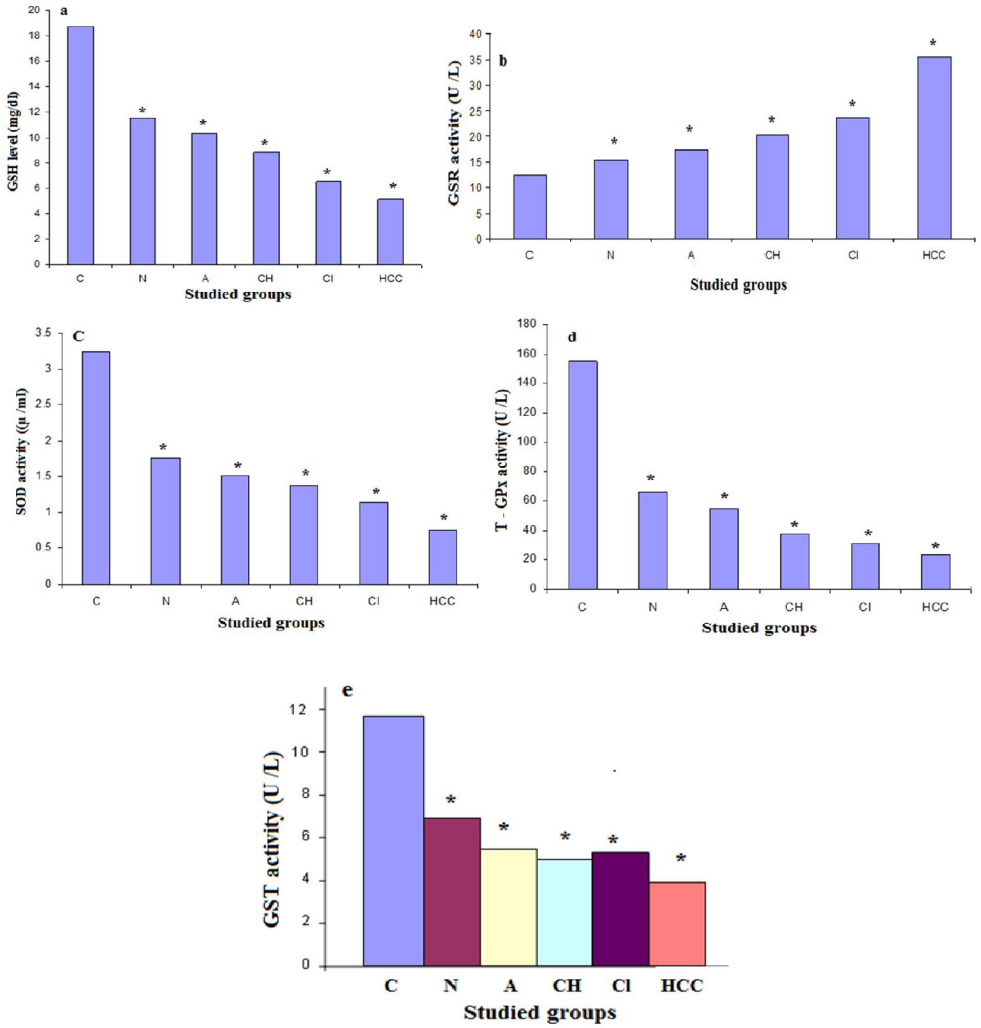

Fig. 3: The levels of antioxidant parameters in different stages of HBV infection.
(a): Serum reduced GSH levels, (b) Serum GSR activities, (c) Serum SOD activities; (c) Serum GPx activities and (e) Serum GST activities. Since C=control samples, N=normal carriers, A=acute infection, CH=chronic infection, Cl= cirrhotic liver and HCC=hepatocellular carcinoma patients.

4. Discussion

In the present study the relationship between HBV DNA titer and different stages of HBV infection was studied and the data showed that there was no significant relationship between them. Our results agree with the previous studies of Yuen *et al.* (27), however, other studies reported that patients with less liver damage had higher viral load (28, 29).

Oxidative stress has been shown to be linked to liver diseases such as hepatotoxicity and other liver pathological conditions (30). In the present study the levels of MDA were elevated in all studied groups and follow the order: HCC > Cl > CH > A > N as compared to the control. This indicates that HBV stimulates the generation of ROS and LPO. It had been reported that ROS act as second messenger molecules and key elements to activate the nuclear factor kapa beta ($NF_K\beta$) in the early events of inflammation. $NF_K\beta$ activates the inducible nitric oxide synthase (iNOS) which produces high amount of NO (31). Yoshie & Oshima (32) reported that NO is considered likely to be an important endogenous factor of carcinogenesis, both by itself and also in association with other agents. Hence, it leads to phagocytosis, amplification of inflammation and tissue injury. In addition, it reacts with $O_2^{-\cdot}$ producing peroxynitrite ($ONOO^{\cdot}$), a powerful oxidant, which damage the biological molecules (33). The generation of O_2, NO and other ROS and reactive nitrogen species (RNS) accelerate peroxidation of native membrane lipids leading to loss of membrane integrity, membrane damage and subsequent release of the cytosolic contents (34). In addition, peroxidation of mitochondrial membrane leads to increase the membrane permeability and alteration of Ca^{2+} homeostasis that contribute to cell death due to alteration in the inner membrane potential (35, 36). Our results are in agreement with the previous studies which reported elevated lipid peroxidation levels in patients with chronic hepatitis B and C (37-39) and in patients with alcoholic liver disease among the chronic alcoholics (40). In addition the previous studies showed that patients with CH, liver cirrhosis (LC), and HCC due to HBV infection had more than 20 times higher urinary epsilond A levels compared to uninfected individuals with no liver disease. Among the mechanisms involved in mediating the process of liver fibrosis, an important role is played by ROS. During the progression of liver injury, hepatic stellate cells (HSCs) become activated, which produce extracellular matrix such as collagen I. Collagen I gene regulation has revealed a complex process involving ROS as a key mediator. ROS-sensitive cytokines contribute to HSC activation during inflammation through paracrine signals released from immune cells. The activated HSCs become responsive to platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β. PDGF facilitates the progression of hepatic fibrosis in human chronic liver diseases (CLD). It increased the accumulation of H_2O_2 in HSCs (7).

Hepatic GSH plays a crucial role in both scavenging ROS and the detoxification of drugs (36). The possible involvement of GSH in the metabolism of such products is indicated by decrease in its level in all studied groups and follows the order: HCC < Cl < CH < A < N as compared with the control. This may be due to the increased OS. This caused insufficient synthesis of GSH to maintain its adequate intracellular levels and in turn led to further GSH depletion (3, 4). This alteration may influence the capability of the liver to provide protection against oxidative damage (41). Also, the decrease in GSH level may be due to its consumption by GST (3, 4). In addition, to GSH depletion in all groups, the activities of both GST and t-GPx enzymes were decreased significantly as compared to the control since they follow the sequence HCC < Cl < CH < A < N. GST and GPx enzymes are GSH dependent (42) so the decline in GSH level may lead to decrease in both enzyme activities. The depression in the levels of both GSH and the activities of GST & t-GPx may be an adaptive response to condition of increased OS. In addition, the decrease in the level of GSH and the activity of GST indicated that the liver had a lower ability to detoxify the ROS resulting in an increased availability of potentially carcinogenic molecules. These carcinogenic molecules targeted hepatocytes to the S-phase of mitosis, eventually leading to a higher number of initiated cells with the time (43).

GSR is an important flavo-enzyme involved with glucose-6-phosphate dehydrogenase (G6PDH) in the maintenance of a reduced intracellular environment. It plays a key role in the cellular defense against oxidative stress by preventing the accumulation of GSSG and thus maintaining the redox state (44). To recycle GSSG, the cell utilizes NADPH-dependent GSR and the NADPH being supplied by G6PDH (45). The levels of hepatic GSH were severely depleted where GSR activity was enhanced in N, A, CH, Cl and HCC groups as compared to the control group. This may be related to the upregulation of G6PDH enzyme which led to increase the level of NADPH leading to elevation of GSR activity (45).

SOD is the first line of defense of the cellular antioxidant system against oxidative damage mediated by superoxide radicals and it is considered as the most effective antioxidant (36). The decline in the activity of SOD in all studied groups in the present study indicates the increased formation of $O_2^{-\cdot}$ radicals in liver tissues. This result is in accordance with other

previous studies (46, 47). The decrease in t-GPx activity may lead to the accumulation of H_2O_2 which in turn feeds back and inhibits SOD (48). This inhibition occurs by oxidation of the cysteine residues in its active site (36). Also, Djordjević (6) reported that the cytosolic CuZn SOD may be inactivated by H_2O_2, leading to the generation of either Cu (II)-OH or its ionized form Cu (II)-O. These products can further catalyze the peroxidation of wide variety of compounds.

The reduction of antioxidant parameters, GSH, GPx, GST and SOD in all studied groups in this study agree with the previous results which reported that these antioxidants are decreased in patients with liver disease of both alcoholic and nonalcoholic etiology (49, 50) and in patients with viral hepatitis (51).

On the other hand, the increase in lipid peroxidation, in the present study led to hepatocytes inflammation, increase in membrane permeability and injury which led to the hepatocytes rupture and leakage of their contents. Therefore TB level and AST and ALT activities were elevated in N, A, CH, Cl and HCC groups as compared to the C group. However the levels of TP and albumin were decreased due to reduction in their biosynthesis. These results agree with the previous results of Lieber (52) and Kew (53).

In conclusion, serum MDA, GSH, antioxidant enzymes and liver function tests measurements are useful in monitoring hepatocellular damage in patients with HBV positive. Also the present study considered that deficiency of antioxidant barrier may cause oxidative stress in patients with HBV and, so antioxidant treatment should be useful for these patients.

References

1- Opara EC. Oxidative stress. Diseases Mon 2006; 52: 183-198.
2- Shaban NZ, Abdul-Aziz AA. Effect of Meloxicam on Lipoxygenase Activity in Trachea, Skin, Stomach and Serum of Rabbits (*in Vivo* and *in Vitro*). Life Science Journal, 2014; 11(6):48-55.
3- Shaban NZ, El-Kersh MA, El-Rashidy, F H, Habashy NH. Protective role of *Punica granatum* (pomegranate) peel and seed oil extracts on diethylnitrosamine and phenobarbital-induced hepatic injury in male rats. Food chemistry 2013; 141(3): 1587-1596.
4- Shaban NZ, El-Kersh MA, Bader-Eldin MM, Kato SA, Hamoda AF. Effect of *Punica granatum* (Pomegranate) Juice Extract on Healthy Liver and Hepatotoxicity Induced by Diethylnitrosamine and Phenobarbital in Male Rats. Journal of Medicinal Food 2014; 17(3): 339-349.
5- Choi J, Ou JH. Mechanisms of liver injury III. Oxidative stress in the pathogenesis of hepatitis C virus. American Journal of Physiology and Gastroenterology ,Liver Physiology 2006; 290: 847-851.
6- Djordjević VB. Free Radicals in Cell Biology. International Review of Cytology 2004; 237: 57-89.
7- Nair J, Srivatanakul P, Haas C, Jedpiyawongse A, Khuhaprema T, Seitz HK, Bartsch H. High urinary excretion of lipid peroxidation-derived DNA damage in patients with cancerprone liver diseases. Mutation Researches 2010; 683: 23-28.
8- Rayman PR. Food-chain selenium and human health: emphasis on intake. British Journal of Nutrition 2008; 100(2):254-268.
9- Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Critical Review of Food Sciences and Nutrition. 2004; 44: 275-295.
10- Chauhan A, Chauhan V. Oxidative stress in autism. Pathophysiology 2006; 13: 171-181.
11- Maser RL, Vassmer D, Magenheimer BS , Calvet JP. Oxidant Stress and Reduced Antioxidant Enzyme Protection in Polycystic Kidney Disease. Journal of American Social Nephrology 2002; 13: 991–999.
12- Vrba J, Modriansky. Oxidative burst of kupffer cells: target for liver injury treatment. Biomedicine Papers 2002; 146(2): 15-20.
13- Rajkapoor B, Murugesh N, Chodon D, Sakthisekaran D. Chemopreventive of N-nitrosodiethylamine induced Phenobarbital promoted liver tumors in rat by extract of *Indigofera aspalathoides*. Biological Pharmacology Bull 2005; 28(2): 364-366.
14- Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. Clinical Microbiology Review 1999; 12:351–366.
15- Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB, Nelson WG. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc National Academic Sciences USA 1997; 91(24):11733-11737.
16- Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annual Review of Immunology 2009; 13:29–60.
17- Wang FS. Current status and prospects of studies on human genetic alleles associated with hepatitis B virus infection. World Journal of .Gastroenterology 2003; 9: 641–644.

18- Atheer AM, Wesen AM, Amani MJ. Study Several Biochemical Parameters into Patient's with Hepatitis B Virus. Global Journal of Medical research Diseases 2013; 13(2): Version 1.0
19- Wang XW, Hussain SP, Huo TI, Wu CG, Forgues M, Hofseth LJ, *et al.* Molecular pathogenesis of human hepatocellular carcinoma. Toxicology 2002; 181(182):43–47
20- Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods in Molecular Biology 1998; 108: 101-106.
21- Hu XM, Hirano T, Oka K. Arsenic trioxide induces apoptosis in MOLT-4 and its daunorubicin-resistant MOLT-4 cell lines *via* depletion of intracellular glutathione, disruption of mitochondrial membrane potential and activation of caspase-3. Cancer Chemotherapeutic Pharmacology 2003; 52:47-58.
22- Michiels C, Raes M, Toussaint O, Remacle J. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radical and Biological Medicine 1994; 17:235-240.
23- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory Clinical Medicine 1967; 70:158-169.
24- Carlberg I, Mannervik B. Glutathione reductase. Methods of Enzymology 1985; 113:484-490.
25- Habig WH, Pabst J, Jakoby B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 1974; 249:7130– 7139.
26- Danil S. Pratt MD, Marshal M. Evaluation of the liver: laboratory tests. Schiff's diseases of the liver, 8th edn. USA; JB Lippincott publications 1999; 205-239.
27- Yuen MF, Oi-Lin Ng I, Fan ST, Yuan HJ, Ka-Ho Wong D, Chi-Hang Yuen J, Siu-Man Sum S, On-On Chan A, Lai CL. Significance of HBV DNA Levels in Liver Histology of HBeAg and Anti-HBe Positive Patients with Chronic Hepatitis B. American Journal of Gastroenterology 2004 ; 99:203-27.
28- Chu CJ, Lok ASF. Clinical utility in quantifying serum HBV DNA levels using PCR assay. Journal of Hepatology 2002; 36: 549-551.
29- Heo J, Baik TH, Kim HH, Kim GH, Kang DH, Song GA, Cho M, Yang US. Serum hepatitis B virus (HBV) DNA levels at different stages of clinical course in patients with chronic HBV infection in an endemic area. Journal of Korean Medical Sciences 2003; 18: 686-90.
30- Minicis S, Brenner DA. Oxidative stress in alcoholic liver disease: role of NADPH oxidase complex. Journal of Gastroenterology and Hepatology 2008; 23:98–103.
31- Ueno S, Aoki D, Kubo F, Hiwatashi K, Matsushita K, Oyama T, Maruyama I, Aikou T. Roxithromycin inhibits constitutive activation of nuclear factor κB by diminishing oxidative stress in a rat model of hepatocellular carcinoma. Clinical Cancer Researches 2005; 11(15): 5645-5650.
32- Yoshie T, Oshima H. Synergistic induction of DNA strands breakage by cateshol-estrogen and nitric oxide: implication for hormonal carcinogenesis. Free Radical and Biological Medicine 1998; 24(2): 341-348.
33- Yen GC, Lai HH. (2002). Inhibitory effects of isoflavones on nitric oxide-or peroxynitrite-mediated DNA damage in RAW 264.7 cells and ΦX174DNA. Food Chemistry and Toxicology 2002; 40(10): 1433-1440.
34- Mittal G, Bar APS, Soni G. Impact of hypercholesterolemia on toxicity of N-nitrosodiethylamine: biochemical and histopathological effects. Pharmacology Reproduction 2006; 58: 413-419.
35- Igbavboa U, Zwizinski GW,Pferiffer DR. Release of mitochondrial matrix proteins through a Ca2+-requiring, cyclosporin-sensitive pathway. Biochemistry and Biophysics Research Communication 1989; 161(2): 619-625.
36- Shaban NZ, Helmy MH, El-Kersh MA, Mahmoud BF. Effects of *Bacillus thuringiensis* toxin on hepatic lipid peroxidation and free-radical scavengers in rats given alpha-tocopherol or acetylsalicylate. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 2003; 135(4): 405-414.
37- Lebensztejn DM, Kaczmarski M, Sobaniec-Lotowska M, Bauer M, Voelker M, Schuppan D. The role of free oxygen radicals in children with chronic viral hepatitis. B. RocznikiAkademiiMedycznej w Bialymstoku 1995; 40(3):667–672.
38- Abd El-Ghaffar Y, Foud HH, Eid A. Effect of interferon-α therapy on oxidative stress in hepatitis C virus infection. Arab Journal of Laboratory Medicine 1999; 25(3):345–354.
39- Yadav D, Hertan HI, Schweitzer P, Norkus EP, Pitchumoni CS. Serum and liver micronutrient antioxidants and serum oxidative stress in patients with chronic hepatitis C. American Journal of Gastroenterology 2002; 97:2634-2649.
40- Chari, Gupta. Status of blood antioxidant enzymes in alcoholic cirrhosis. Indian Journal of

Physiology and Pharmacology 2003; 47 (3): 343 – 346.
41- Czeczot H, Ścibior D, Skrzycki M, Podsiad M. Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. Acta Biochemica Polonica 2006; 53(1): 237-241.
42- Scanlan N. Compromised hepatic detoxification in comparison animals and its correction via nutritional supplementation and modified fasting. Alternative Medicine Review 2001; 6: 24-37.
43- Muzio G, Marengo B, Salvo R, Semeraro A, Canuto RA, Tessitore L. Liver cancer is induced by a subnecrogenic dose of DENA when associated with fasting/refeeding: role of glutathione-transferase and lipid peroxidation. Free Radical and Biological Medicine 1999; 26: 1314-1320.
44- Muller JG, Bucheler US, Kayser K, Schrimer RH, Werner D, Krauth-Siegel R. Glutathione reductase in human and murine lung tumors: high levels of mRNA and enzymatic activity. Cell and Molecular Biology 1993; 39(4): 389-396.
45- Salvemini F, Franzé A, Iervolino A, Filosa S, Salzano S ,Ursini, MV. Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. Journal of Biological Chemistry 1999; 274: 2750-2757.
46- Irshad M, Chaudhuri PS, Joshi YK (2002) Superoxide dismutase and total anti-oxidant levels in various forms of liver diseases. Hepatol Res 23:178-184.
47- Ramakrishnan G, Raghavendran HR, Vinodhkumar R, Devaki T. Suppression of N-nitrosodiethylamine induced hepatocarcinogenesis by silymarin in rats. Chemistry and Interactation 2006; 161: 104-114.
48- Kakkar R, Mantha S, Radhi J, Prasad K, Karla J. Antioxidant defense system in diabetic kidney. Life Science 1997; 60: 667-679.
49- OEwiêtek K, Juszczyk J. Reduced glutathione concentration in erythrocytes of patients with acute and chronic viral hepatitis. Journal of Viral Hepatitis 1997; 4: 139–141.
50- Demirdag K, Yilmaz S, Ozdarendeli A, Ozden M, Kalkan A, Kilic SS. Levels of plasma malondialdehyde and erythrocyte antioxidant enzyme activities in patients with chronic hepatitis B. Hepatogastroenterology 2003; 50:766-770.
51- Osman HG, Gabr OM, Lotfy S, Gabr S. Serum levels of bcl-2 and cellular oxidative stress in patients with viral hepatitis. Indian Journal of Medical Microbiology 2007; 25:323-329.
52- Lieber CS. Alcohol and liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. Journal of Medicine 2000; 67(1): 84-94.
53- Kew MC. Serum aminotransferase concentration as evidence of hepatocellular damage. Lancet 2000; 355:591–592.

5/21/2014