ISSN : 0974 - 7532 Volume 7 Issue 11

Research & Reviews in

BioSciences

Regular Paper

RRBS, 7(11), 2013 [467-472]

Biochemical alterations in hepatocellular carcinoma patients treated with doxorubicin

Saber A.Sakr*, Nahed S.Bassely[2], Mona A.Sakr[3], Mahmoud Moawad[3], Hassan M.Ahmed[2]

[1]Zoology Department, Faculty of Science, Menoufia University, Shebin El-kom, (EGYPT)

[2]National Organization for Drug control and Research, Cairo, (EGYPT)

[3]National Cancer Institute, Cairo University, Cairo, (EGYPT)

E-mail: sabsak@yahoo.com

ABSTRACT

Doxorubicin (Dox) is an anthracycline antibiotic used as a single chemotherapeutic agent for HCC and has been shown to produce a response rate of about 10-15% but with no proven survival benefits. The present work was conducted to study the biochemical alterations in HCC patients treated with doxorubicin. The study included 30 patients with a confirmed diagnosis of hepatocellular carcinoma (HCC). They were divided into 3 groups. Group 1.Ten specimens of heptocellular carcinoma patients were taken before doxorubicin treatments. Group 2.Ten specimens of heptocellular carcinoma patients were taken one week after doxorubicin treatment. Group 3.Ten specimens of heptocellular carcinoma patients were taken two weeks after doxorubicin treatment. Another ten normal volunteers were used as controls. Treatment schedule consists of i.v. injection of doxorubicin at a dose of 15 mg/m^2 weekly for 3 weeks. Ascitic fluid and blood serum were collected for biochemical examinations. The results showed that ascitic AFP, CEA, T.LDH and LDH4, LDH5 increased significantly in HCC patients. ALT, AST and ALP showed significant increase in sera of HCC patients. These biochemical parameters revealed significant decrease in Dox treated patients.

KEYWORDS

Hepatocellular carcinoma;
Doxorubicin;
Patients;
Biochemistry.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancer worldwide. It accounts for more than 90% of all primary hepatic tumors[9] its incidence increases with age and is five times more common in men than in women[32]. HCC occurs as a complication of hepatic cirrhosis due to various etiologies. Early incidence of HCC in patients with cirrhosis is estimated to be around 3% to 5%[24]. The hyperendemicity of hepatitis B virus infection in Africa and Asia explains the higher incidence of HCC in these regions compared to western countries[29]. Nonetheless, the incidence of HCC in western countries is expected to increase owing to the high prevalence of hepatitis C virus infection that represents a high risk factor for HCC[15].

The most commonly used single agents for HCC are the anthracyclines and anthraquinones, doxorubicin[4,12], 4'- epidoxorubicin[11], and mitoxantrone. Doxorubicin (Dox) is the first generation anthracycline anti-

Regular Paper

biotic of wide spectrum of action. At the cellular level, it is incorporated inbetween two nitric bases of double DNA helix, thus causing the inhibition of DNAdependent DNA and RNA polymerases. This results in the suppression of DNA and RNA synthesis and damage to DNA repair mechanisms[20]. Dox is used as single chemotherapeutic agents and showed a consistent response rate of more than 10%[17]. Nerenstone et al.[23] reported that the overall response rate is approximately 20%, with a median survival of 4 months. However, in a systematic review of five other randomized trials of doxorubicin therapy, no significant survival effect was discernable. The present work aims to investigate the biochemical changes in hepatocellular carcinoma patients treated with doxorubicin.

MATERIALS AND METHODS

Patients

The present study was performed at the National Cancer Institute, Cairo University,Cairo, Egypt. The study met the criteria of the Ethics Committee of the Institution. The study included 30 cases (24 males and 6 female) had a confirmed diagnosis of hepatocellular carcinoma (HCC). They were divided into 3 groups.

Group 1

Ten specimens of heptocellular carcinoma patients were taken before doxorubicin treatments.

Group 2

Ten specimens of heptocellular carcinoma patients were taken one week after doxorubicin treatment.

Group 3

Ten specimens of heptocellular carcinoma patients were taken two weeks after doxorubicin treatment. Another ten normal volunteers were used as controls.

Treatment schedule

Treatment schedule consists of i.v. injection of doxorubicin at a dose of 15 mg/m^2 weekly for 3 weeks. The cycle is repeated as long as the condition permits and the total dose of 500 mg/m^2 are not exceeded.

Biochemical investigations

Ascitic fluid samples were aspirated for estimation of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) and lactate dehydrogenase (T.LDH) and its isoenzymatic activities (LDH4, LDH5). Serum was used for determination of alkaline Phosphates (ALP), aspirtate aminotransferase (AST) and alanine aminotransferase (ALT). AFP and CEA were determined utilizing the kit of Chemiluminescence Immunoassay, based on Tietz[34] and Abeelev (1974), respectively. T.LDH and LDH4, LDH5 activities were determined according to the method of Thomas and Hens[33]. ALP was determined according to the method of Belfied and Goldberge[2]. Colorimetric determination of serum ALT and AST activities was done according to the method of Reitman and Frankel[25].

Statistical analysis

Sigma plott system was used for data analysis. Mean and standard error were used as descriptive measures. ANOVA was used for comparing means of independent groups. P value is significant if<0.05.

RESULTS

The mean age of patients was 56.7 ± 7.71, 65.1± 12.1, 63±13.4 and 58.6± 12.5 years in control cases, HCC group, HCC treated with Dox for 1 week and HCC treated with Dox for 2, respectively.

Biochemical results

Ascetic AFP and CEA.

Data regarding AFP of all groups were summarized in figure 1. In control cases, ascitic AFP showed a mean of (4.6 ± 3.9 ng / ml). In HCC before treatment, ascitic

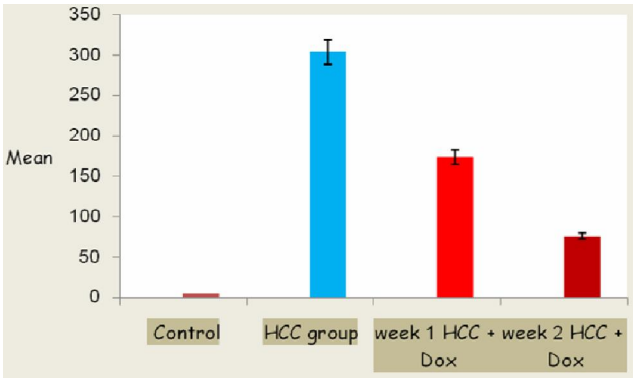

Figure 1 : Ascitic AFP (*ng / ml*) in the studied cases.

AFP showed a mean of (303.7 ± 128.7 ng / ml). On other hand, the patients after one week of treatment showed a mean (174.5 ± 91.6 ng / ml), but the patients after two weeks of treatment showed a mean (76.2 ± 40.1 ng / ml). Ascetic CEA showed statistically signifi-

Regular Paper

cant increase in malignant group compared to the cases which used as control group (2.7 ± 1.8 ng / ml). The highest contribution was from the HCC before treatment which showed statistically significant increase (12.5 ± 4.7 ng/ml) compared to all other groups. The lower value was from the HCC after two weeks of treatment which showed statistically significant decrease (4.55 ± 2.11 ng /ml) compared to all other groups (figure 2).

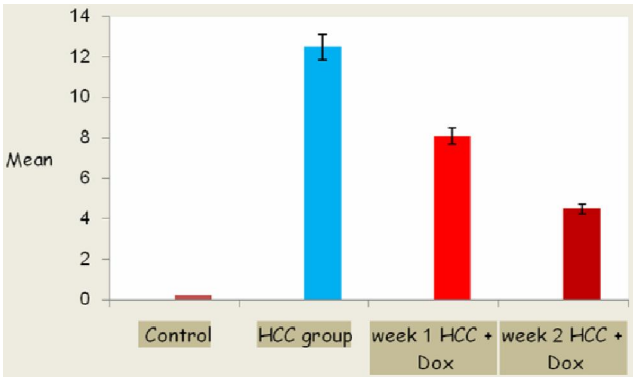

Figure 2 : Ascitic CEA (*ng / ml*) in the studied cases.

Ascetic lactate dehydrogenase and its isoenzymatic activities

Data regarding LDH and its isoenzymes of malignant and control cases were summarized in TABLE 1. Ascetic T.LDH and LDH4, LDH5 showed statistically significant increase in malignant group compared to the control cases. HCC cases before treatment showed statistically significant increase compared to all other groups. But the lower contribution was from the HCC after two weeks of treatment which showed statistically significant decrease compared to all other groups.

TABLE 1 : Change in total lactate dehydrogenase (T.LDH) and its isoenzymatic activities in different cases

Treatment	T.LDH	LDH4	LDH5
Control	1.3±0.3	0.5±01	0.3± .1
HCC group	196.5±27.9	26.6±3.7	21.7±2.1
HCC+1weekDox	154.5±16.5*	23.9±3.2	20.6±2.1
HCC+2weekDox	97.8±23.8**	21.2±5.0*	14.8±6.7**

(*) Significant at P<0.05, (**) Significant at P<0.001

Serum ALP, ALT and AST

In control groups, serum ALT and AST showed means of (32.7 ± 5.9 U / L) and (41.1 ± 9.8 U / L), respectively. These values showed a significant increase in HCC groups and the means of ALT and AST were (273.9 ±9.4 U / L) and (342.0 ± 45.3 U / L), respectively. On other hand, the activities of these enzymes decreased significantly in patients treated with Dox for two weeks (figures 3&4). Serum ALP showed statistically significant increase in HCC group compared to control cases. The activity of ALP significantly decreased after two weeks of HCC treatment (Figure 5).

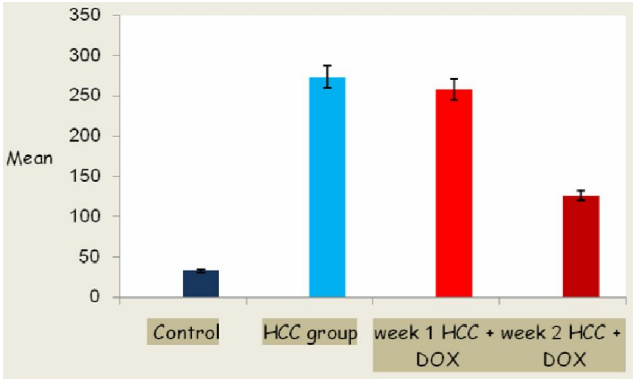

Figure 3 : Serum ALT (*U/ L*) in the studied cases.

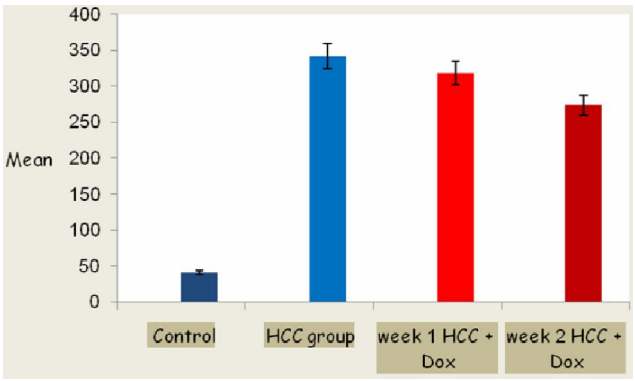

Figure 4 : Serum AS T (*U/L*) in the studied cases.

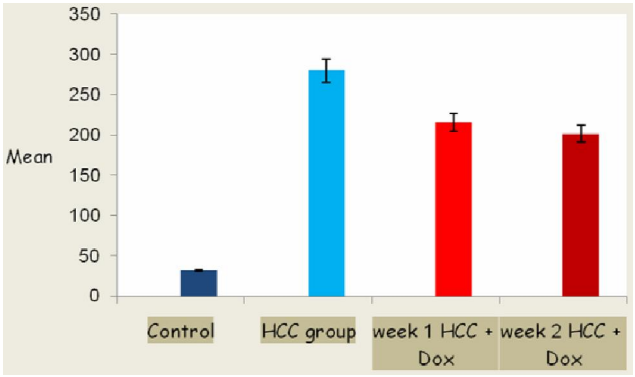

Figure 5 : Serum ALP (*U/ L*) in the studied cases.

DISCUSSION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide[30], ranks fifth in frequency worldwide among all malignancies and the third most common cause of cancer-related death. It causes one million deaths annually[18]. HCC comprises clinically che-

Regular Paper

motherapy resistant tumors, and this observation is supported by low response rates across a wide variety of cytotoxic agents. The most widely used agent has been doxorubicin, both as a single agent and in combination with other drugs. An early randomized trial against best supportive therapy showed slightly increased survival, in the order of weeks[23].

In the obtained result, ascitic AFP showed statistically significant increase in HCC group compared to control cases. This in agreement with Gadelhak et al.[7] who reported that concentration of AFP was greater than the upper reference limit indicate the presence of HCC, but values below this level are less useful because they may also occur in chronic liver disease. AFP is the most popular tumour marker for hepatocellular carcinoma (HCC). It is used in diagnosis and follows up of cases by estimating its rise. It was found that serum AFP level was elevated in 72.7 % of HCC patients, and in ascitic fluid was elevated in 63.6 %. Also, there was a highly significant, positive correlation between elevation of AFP in serum and in ascitic fluid (r = 0.778). Elevation of AFP in ascitic fluid is of high importance in evaluation of HCC and is as significant as serum and runs parallel to it. Estimation of AFP in ascitic fluid is much more significant in evaluation of HCC cases than ascitic fluid cytology[27]. This protein can directly diffuse from the liver towards the peritoneal cavity[36] and have molecular weight, around 70 kD. Thus, based on a possible release of AFP by the hepatic tumour directly into the ascitic fluid, Khakoo et al.[15] made the hypothesis that ascitic fluid AFP might have a better diagnostic value than serum AFP. A significant decrease in AFP was recorded in Dox-treated patients. Malagari et al.[19] reported that AFP levels decreased significantly in measurements 1 month post Dox-chemotherapy of HCC. Yau et al.[37] recorded that AFP response was defined as a relative drop of AFP >20% of the baseline level after sorafenib treatment. AFP-producing capacity of hepatoma cells can be changed by chemotherapeutic agents, probably through chromosomal mutation[22].

A significant increase in ascitic CEA was recorded in HCC patients compared with control cases. showed statistically significant increase in malignant group compared to the cases which used as control group. Sell[28] showed that CEA levels elevate in some patients with pathological conditions such as cirrhosis (45%), pulmonary emphysema (30%), rectal polyps (5%), benign breast disease (15%), and ulcerative colitis (15%). Recent evidence suggests that CEA is a cellular adhesion molecule that may potentiate invasion and metastasis. In this study, ascetic CEA level showed significantly decrease after Dox treatment. This study was recorded by Ku et al.[16] who said that a sharp decrease in serum CEA levels (to < 50% of their pretreatment levels) after Dox treatment.

The present results revealed significant higher levels of ascitic T.LDH in the HCC group compared to the control group. These results were in agreement with those of Gerbes et al.[8] and Castaldo et al.[3]. Glannoulaki et al.[10], showed that serum total LDH activity significantly increased in the non- malignant group and the malignant group compared to the control group. Ascetic LDH isoenzymes (LDH4 and LDH5) showed statistically significant increase in HCC group compared to the control cases. Zondag and Klein (1968) reported that one of the best characterized features of tumor growth is the associated alteration in the enzyme and isoenzyme pattern of tissues in the host organs. LDH is one of the enzyme system preferentially produced and retained by cancer cells, being necessary to maintain tumor growth. When LDH isoenzymes are released from neoplastic tissue in serum, the LDH isoenzymes pattern of the serum changes. Wilkinson[35] reported that elevation in LDH4 and LDH5 in malignant cases is due to re-emergence of foetal pattern of isoenzyme distribution i.e. to anaerobic (cathodal) isoenzyme which is a feature malignant transformation in many tissues. Ascetic T.LDH, LDH4 and LDH5 showed a significant decrease after Dox treatment. Similarly, Sun et al.[31] reported that LDH slowly decreased in HCC patients treated with tamoxifen, suggesting a slower, but continuous, progression of the disease after tamoxifen treatment.

Serum ALT and AST showed statistically significant increase in HCC group compared to control cases. This result was in agreement with Rosalki[26] who recorded that the AST and ALT levels are increased to some extent in almost all liver diseases such as viral hepatitis, drug or toxin induced hepatic necrosis and circulatory shock. Elevated plasma activities of AST or ALT are regarded as sensitive indicator of liver cells damage[5]. In this work, the lowest contribution of ALT and AST was from the HCC after Dox treatment. In

agreement of this result, Kazuhiro et al.[14] showed that administration of rifampicin markedly decreased ALT and AST values in HCV-related liver cirrhosis patients with a high risk of HCC. Rifampicin has an anti-inflammatory effect on hepatocyte disorder by preventing the release of hepatic enzymes, including ALT and AST, and indirectly suppresses liver injury by inhibiting secretion of cytokines.

In present result, ALP showed statistically significant increase in HCC group compared to control cases.. It has been recognized that a raised ALP level in the absence of jaundice, may be used as possible presumptive evidence of HCC[13]. The activity of ALP decreased after two weeks of HCC treated with Dox. This result agrees with Mulders et al.[21] who reported a decrease in ALP during the first months of chemotherapy treatment in HCC cases.

REFERENCES

[1] G.I.Abeelev, S.D.Perova, N.I.Khramkova, Z.A.Postnikova, I.S.Irlin; Production of embryonal alpha-globulin by transplantable mouse hepatomas. Transplantation, **1**, 174–80 **(1963)**.

[2] A.Belfied, D.M.Goldberg; Revised assay for serum alkaline phosphatase activity using 4 – amino – antipyrine. Enzyme, **12(5)**, 561-573 **(1971)**.

[3] G.Castaldo, G.Oriani, L.Cimino, M.Topa, I.Mostarda, L.Castellano, C.Del-Vecchio-Blouco, L.Sacchetti; Total discrimination of peritoneal malignant ascities from cirrhosis and HCC-associated ascites by assay of ascetic cholesterol and lactate dehydrogenase. Clin. Chem, **4(3)**, 478-84, Mar **(1994)**.

[4] R.T.Chlebowski, A.Brzechwa-Adjukiewicz; Doxorubicin (75 mg/m2) for hepatocellular carcinoma: Clinical ad pharmacokinetic results. Cancer Treat Rep., **68**, 487-491 **(1984)**.

[5] R.J.Clermont, T.C.Chalmers; The transaminase tests in liver disease. Medicine, **46**, 197-207 **(1967)**.

[6] A.Dunk, S.C.Scott, P.J.Johnson; Mitoxantrone as single agent therapy in hepatocellular carcinoma: a phase II study. J Hepatol, **1**, 395-404 **(1985)**.

[7] N.A.Gadelhak, S.A.Gadelhak, D.A.El-Morsi, M.M.Abdelaziz, A.T.Abbas, H.M.El-Emshaty; Prognostic significance of three hepatitis markers (p53 antibodies, vascular endothelial growth factors and alpha fetoprotein) in patients with hepatocellular carcinoma. Hepatogastroenterology, **56(94-95)**, 1417-24 **(2009)**.

[8] A.L.Gerbes, D.Jungst, Y.Xie, W.Permanetter, G.Paumgartner; Ascitic fluid analysis for the differentiation of malignancy-related ascites and non-malignant ascites. Cancer, **68**, 1808–14 **(1991)**.

[9] G..Giannelli, C.Bergamini, E.Fransvea; Human HCC cells require both alpha3 B, lntegrin and Matrix metallooroteinases activity for migration and invasion. Laboratory investigation, **81(4)**, 613-627 **(2001)**.

[10] E.E.Glannoulaki, D.L.Kalpaxis, G.Tents, P.Fessal; Lactate dehydrogenase iso-enzymatic pattern in sera of patients with malignant disease. Clin.Chem, **35**, 396-9 **(1989)**.

[11] H.Hochster, M.Green, J.Speyer; 4'-Epidoxorubicin (epirubicin): Activity in hepatocellular carcinoma. J Clin Oncol, **11**, 1535-1540 **(1985)**.

[12] P.J.Johnson, H.Thomas, R.Williams; Induction of remission in hepatocellular carcinoma with doxorubicin. Lancet, **1**, 1006-1009 **(1987)**.

[13] P.J.Johnson; Role of the standard liver function test in current clinical practice. Ann Clin Biochem, **29**, 463-71 **(1989)**.

[14] K.Kazuhiro, K.Yutaka, S.Masanobu, F.Yasuhiro, S.Masayoshi, T.Yujiro; Hepatocyte-protective and anti-oxidant effects of rifampicin on human chronic hepatitis C and murine acute hepatocyte disorder. Experimental and Therapeutic Medicine, 1041-1047 **(2010)**.

[15] S.I.Khakoo, L.F.L.Grellier, S.Bhattcharya, M.Dusheiko; Aetiology, screening and treatment of hepatocellular carcinoma. Med Clin North Am, **80**, 1121–45 **(1996)**.

[16] Y.Ku, T.Iwasaki, T.Fukumoto, M.Tominaga, S.Muramatsu, N.Kusunoki, T.Sugimoto, Y.Suzuki, Y.Kuroda, Y.Saitoh; Percutaneous isolated liver chemoperfusion for treatment of unresectable malignant liver tumors: technique, pharmacokinetics, clinical results. Cancer Res., **147**, 67-71 **(1998)**.

[17] C.L.Lai , P.C.Wu, G.C.Chan, A.S.Lok, H.J.Lin; Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A prospective randomized trial. Cancer, **62(3)**, 479-483 **(1988)**.

[18] J.M.Liovet, J.Bruix; The Barcelona Approach: diagnosis, staging, and treatment of hepatocellular carcinoma. Liver transplantation, **10(2)**, S115-S120 **(2004)**.

[19] K.Malagari, E.Alexopoulou, K.Chatzimichail, B.Hall, J.Koskinas, S.Ryan, E.Gallardo, A.Kelekis, A.Gouliamos, D.Kelekis; Transcatheter chemoembolization in the treatment of HCC in pa-

Regular Paper

tients not eligible for curative treatments: midterm results of doxorubicin-loaded DC bead. Abdom Imaging, **33(5)**, 512-9 **(2008)**.

[20] R.L.Momparler, M.Karon, S.E.Siegel, F.Avila; Effect of adriamycin on DNA, RNA and protein synthesis in cell-free systems and intact cells. Cancer Res, **36**, 2891-2895 **(1976)**.

[21] P.F.Mulders, Moral P.Fernandez del, A.G.Theeuwes, G.O.Oosterhof, H.T.van Berkel, F.M.Debruyne; Value of biochemical markers in the management of disseminated prostatic cancer. Eur Urol, **21(1)**, 2-5 **(1992)**.

[22] A.Muraoka, T.Tokiwa, J.Sato; Alpha-fetoprotein-producing capacity, chromosomal and morphological properties in human hepatoma cells treated with various chemotherapeutic agents. Research in Experimental Medicine, **189(6)**, 409-19 **(1989)**.

[23] S.R.Nerestone, D.C.Ihde, M.A.Friedman; Clinical trials in primary hepatocellular arcinoma: Current status and future directions. Cancer Treat Rev, **15**, 1-31 **(1988)**.

[24] W.H.Ramsey, G.Y.Wu; Hepatocellular carcinoma: update on diagnosis. Dig Dis Sci, **33**, 1601-1614 **(1995)**.

[25] S.Reitmn, S.Frankel; Acolorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyrovic transaminase. Am. J. Clin. Pathol, **28(1)**, 56-63 **(1957)**.

[26] S.B.Rosalki, N.Mcintyre; Biochemical investigations in the management of liver disease. Oxford textbook of clinical hepatology, 2nd ed. New York; Oxford University press, 503-521 **(1999)**.

[27] A.Sadek, A.Abd EL-Hady; Alpha feto- protein and albumin in ascetic fluid in hepatocellular carcinoma patients: J. Egypt. Soc. Parasitol, **27(2)**, 455- 64 **(1997)**.

[28] S.Sell; Serelogical Cancer Markers. Totowa, N.J.Humana Press, **(1992)**.

[29] R.G.Simonetti, C.Camma, F.Fiorello, F.Politi, D'G.Amico, L.Pagliaro; Hepatocellular carcinoma: a world-wide problem and the major risk factors. Dig Dis Sci, **36**, 962–72 **(1991)**.

[30] M.C.Stern, M.David, M.Yu, B.Hepatitis; Aflatoxin-B1. And P53 codon 249 mutation in HCC from Guangxi, people republic of China and a meta-analysis of existing studies. Cancer pidemiology Biomarkers and Prevention, **10**, 617-625 **(2001)**.

[31] H.Sun, L.Yu, H.Wei, G.Liu; A novel antihepatitis drug, bicyclol, prevents liver carcinogenesis in diethylnitrosamine-initiated and phenobarbital-promoted mice tumor model. J. Biomed. Biotechnol. Epub, **(2012)**.

[32] D.Tavian, G.De Petro, A.Pitozzi; Androgen receptor mrna under-expression in poorly differentiated human hepatocellular carcinoma. Histol Histopathol, **17(4)**, 1113-1119 **(2002)**.

[33] P.Thomas, D.A.Hens; The hepatic clearance circulatory native and asialo Carcinoemberionic Antigen. Biochem. Biophys.res.Cemmu, **67**, 1205-1209 **(1975)**.

[34] N.W.Tietz; Fundanental of Clinical Chemistry. W.B. Saunders Co., Philadephia, **(1996)**.

[35] J. H.Wilkinson; Clinical applications of isoenzymes. In Topics in Medicinal Chemistry, Wiley, New York, **(1970)**.

[36] M.H.Witte, C.L.Witte, A.E.Dumont; Estimated net transcapillary water and protein flux in the liver and intestine of patients with portal hypertension from hepatic cirrhosis. Gastroenterology, **80**, 265–72 **(1981)**.

[37] T.Yau, T.J.Yao, P.Chan, H.Wong, R.Pang, S.T.Fan, R.T.Poon; The significance of early alpha-fetoprotein level changes in predicting clinical and survival benefits in advanced hepatocellular carcinoma patients receiving sorafenib. Oncologist, **16(9)**, 1270-9 **(2011)**.

[38] H.A.Zandage, F.Klein; Clinical applications of lactate dehydrogenase isoenzymatic alterations in malignant. Ann.N.Y.Acad.Sci., **151**, 578-586 **(1978)**.