International Journal of Tropical Medicine 2 (3): 101-106, 2007
ISSN: 1816-3319

State of Liver Metabolic Function after Dimethyl Diphenyl Bicarboxylate Treatment in HCV Patients Using Antipyrine Clearance in Comparison to Conventional Liver Function Tests

Sanaa Botros, Rania Abdel-Kader, Maged El-Ghannam,
Ahmed El-Ray, Samira Saleh and Madiha Mahmoud
Department of Pharmacology and Hepato-Gastroentrology,
Faculty of Pharmacy,
Theodor Bilharz Research Institute, Warrak ElHader,
PO.Box 30 Imbaba, Giza, Egypt

Abstract: This study investigates the effect of dimethyl diphenyl bicarboxylate, which is widely used in Egypt, as a treatment for hepatitis C patients on the metabolic or biochemical liver function, using antipyrine clearance test and conventional liver function tests. All subjects ingested 600 mg antipyrine after overnight fasting. One milliliter saliva was collected and pharmacokinetic values were examined using high performance liquid chromatography according to first kinetic order. Blood samples were collected for examination of conventional liver function tests. All parameters were examined at the onset of the study and at 2, 6 and 12 weeks post dimethyl diphenyl bicarboxylate treatment. Thirty three hepatitis C patients complying to treatment follow-up protocol were compared to 15 healthy volunteers. Child-Pugh class B and C patients did not show any improvement in either antipyrine clearance or biological parameters, yet they were limited in number. Child-Pugh class A comprising 20 patients still did not show any improvement in antipyrine clearance, aspartate aminotransferase and gamma-glutamyl transferase, apart from significant improvement in alanine aminotransferase. Therefore, it can be concluded that dimethyl diphenyl bicarboxylate did not improve the metabolic or biochemical liver function that was impaired due to hepatitis C virus infection.

Key words: Dimethyl Diphenyl Bicarboxylate (DDB), Antipyrine Clearance (APC), Hepatitis C Virus (HCV), patients, child-pugh classification

INTRODUCTION

Many lines of treatment of Hepatitis C Virus (HCV) infection have been tried including, recombinant alpha interferon (Rasi *et al*., 1996), interferon-ribavirin combination (McHutchison *et al*., 1998) and Pegylated alpha interferon (Zeuzem *et al*., 2000). Now the Pegylated interferon-ribavirin combination is the standard therapy for treatment of chronic active HCV (NIH, 1998), but interferon therapy is expensive and has many side effects. Other lines such as alternative medicines although not curative were recommended (Salama *et al*., 2004). Peyton *et al*. (1999) stated that 36% of patients with chronic HCV were taking herbal preparations as complementary and alternative medicine.

Dimethyl Diphenyl Bicarboxylate (DDB) is a synthetic mimic of the natural product schisandrin C, a component of Fructus *Schizandrae* (Shen *et al*., 1987). It has been registered as liver support medication in China (Lee, 2000) and it is currently used for the treatment of chronic viral hepatitis B and C in Asia e.g., China, Korea, Vietnam, Indonesia, Pakistan, Burma (Sun and Liu, 2005) and Egypt (Salama *et al*., 2004; Montasser, 2001). DDB was reported normalization of elevated Alanine Aminotransferase (ALT) levels in patients with chronic liver diseases (Liu, 1987), while Aspartate Aminotransferase (AST), Gamma-Glutamyl Transferase (GGT) and glutamate dehydrogenase levels were not affected (Huber *et al*., 2004).

As estimates of conventional liver function tests in patients under DDB treatment were conflicting, it was essential to look for alternative hepatic functional assay. Antipyrine Clearance (APC) was selected to examine the effect of DDB, because APC represents the intrinsic

Corresponding Author: Madiha Mahmoud, Department of Pharmacology, Theodor Bilharz Research Institute, El-Nile St., Warrak El-Hader, P.O.Box 30, Imbaba, Giza, Egypt

hepatic clearance due to its low binding to plasma proteins and low hepatic extraction ratio (St Peter and Awni, 1991). APC is a dynamic evaluation, contrary to conventional liver function tests which are considered static assays (Sturgill and Lambert, 1997) and it has been used effectively in testing the effect of other drugs or diseases on drug-metabolizing enzymes in the liver (St Peter and Awni, 1991).

This study investigates the effect of DDB as a treatment for HCV patients using APC test as a hepatic metabolic function in comparison to conventional liver function tests.

MATERIALS AND METHODS

The study protocol was approved by the Theodor Bilharz Research local ethical committee for the protection of human rights and patients were admitted to the hepato-gastroentrology department. All patients gave informed written consent and their eligibility was determined by medical history, physical examination and laboratory tests. Subjects were excluded for hypersensitivity to antipyrine and/or ingestion of known powerful enzyme inducers or inhibitors within the past 90 day. Patients were divided into 3 classes (A, B and C) according to Child-Pugh *et al.* (1973). DDB (Beijing Union Pharmaceutical Factory, P.R. China) was given in a dose of 15 mg [10 pills of 1.5 mg DDB/pillule] three times daily for 3 months. Subjects were then subjected to analysis of their APC and liver functional tests at the onset of the study (0-week), 2, 6 and 12 weeks post treatment.

Antipyrine clearance test: All subjects ingested 600 mg of antipyrine (Mehta *et al.*, 1986) in the form of hard gelatinous capsule after overnight fasting and then 1 mL of saliva was collected at 4 and 24 h and kept frozen at -70°C. Antipyrine was extracted (Echnizen *et al.*, 1990), analysed using High Performance Liquid Chromatography (HPLC) [Waters, USA] and Novapak C18 (100×5 mm) column (Teunissen *et al.*, 1983). The antipyrine and internal standard mixture were detected at 254 nm on programmable multi-wavelength detector of HPLC (Waters Assoc, Milford, MA, USA).

Conventional liver function test: Five milliliter blood were collected and serum was separated for estimation of INR of prothrombin time (Hougie, 1982), ALT, AST (Reitman and Frankel, 1957), GGT (Persijn and Van der Slik, 1976), total serum bilirubin (Jendrassik and Grof, 1938), Alkaline Phosphatase (ALP) (DGKC, 1972), total proteins (Gornall *et al.*, 1949) and albumin (Bartholomew and Delany, 1964). Globulins and the ratio of Albumin to Globulins (A/G ratio) were calculated.

Data analysis: Pharmacokinetic values for antipyrine was determined according to 1st kinetic order (Poulsen and Loft, 1988) using 2 concentration time points 4 and 24 h as a 1 compartment simple model system (Marques *et al.*, 2002). The saliva concentration versus time curves was plotted to calculate the clearance parameters.

Statistical analysis: Statistical analysis was performed using the statistical program Graphpad Prism and $p<0.05$ was considered statistically significant. Comparisons were performed using t-test for homogenous data and Mann-Whitney test for non-homogenous data. by comparing all Child-Pugh classes' values before treatment to normal controls. If parameter is significantly different from normal, significance was then examined between each follow-up observation values and basal (0-week) values.

RESULTS

Normal volunteers comprised 4 (27%) males and 11 (73%) females. Their age ranged from 25-52 years old. HCV patients were 96 and those complying to treatment protocol were 33 (20 Child-A, 6 Child-B and 7 Child-C), 19 were males and 14 females.

Child-A patients, results presented are that of 20 Child-A patients complying to follow-up treatment protocol except for one patient who completed only 2 weeks follow-up after treatment. Patients showed significantly ($p<0.001$) less values of APC when compared to normal volunteers. After treatment with DDB patients did not reveal any significant improvement in APC 2, 6 and 12 weeks after treatment (Table 1 and Fig. 1).

Child-B patients complying to follow-up protocol after treatment were 6. Only 2 patients completed 6 weeks follow-up after treatment. Compared to normal volunteers, Child-B patients revealed significantly ($p<0.001$) less APC.

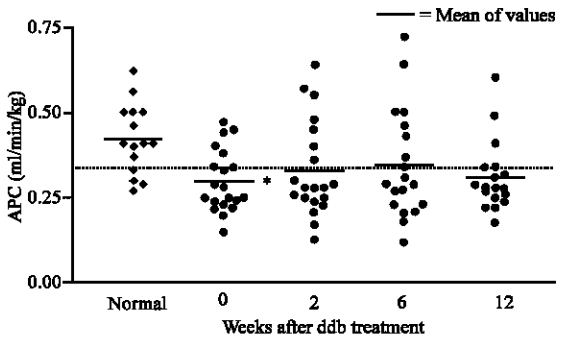

Fig. 1: Antipyrine clearance in normal volunteers and Child-Pugh class A HCV patients before (0 week) and at different observation periods post DDB treatment

Table 1: Liver function tests in serum of hepatitis C patients of Child-A class before treatment and at different observation periods after DDB therapy

Item	Normal volunteers (15)	Weeks post DDB treatment 0 (20)	2 (20)	6 (19)	12 (19)
APC (ml/min/kg)	0.42	0.30 a	0.33	0.35	0.31
	± 0.03	±0.02	±0.03	±0.04	±0.02
ALT (U mL^{-1})	19.53	57.55 a	23.00 b	23.63 b	22.05 b
	±2.53	±8.69	±3.21 b	±3.44 b	±2.81 b
AST (U mL^{-1})	29.67	52.70 a	37.90 b	44.32	48.74 a
	±2.63	±8.57	±5.84	±6.15	±6.84
GGT (U L^{-1})	21.13	59.25 a	72.60 ab	55.11 a	58.32 a
	±1.80	±10.59	±11.22	±9.01	±8.52
ALP (U L^{-1})	130.80	135.10	125.90	108.58	108.10
	±10.9	±13.92	±13.37	±12.30	±11.69
Bilirubin (mg dL^{-1})	0.81	1.03	0.99	0.96	0.97
	±0.06	±0.10	±0.10	±0.11	±0.06
INR	1.21	1.32 a	1.34	1.34	1.33
	0.03	±0.05	±0.04	±0.04	±0.04
Total proteins (g dL^{-1})	7.60	7.63	7.33	7.43	7.24
	± 0.13	±0.13	±0.19	±0.14	±0.15
Albumin (g dL^{-1})	4.17	4.03	3.66	3.62	3.73
	±0.13	±0.13	±0.12	±0.11	±0.13
Globulins (g dL^{-1})	3.43	3.60	3.67	3.81	3.51
	± 0.13	±0.16	±0.17	±0.15	±0.17
A/G ratio	1.28	1.18	1.05	0.98	1.13
	± 0.08	±0.08	±0.07	±0.06	±0.08

Values are means ± SEM, ( ) Number of patients or volunteers, APC: Antipyrine Clearance, ALT: Alanine aminotransferase AST: Aspartate aminotransferase GGT: Gamma Glutamyl Transferase, ALP: Alkaline Phosphatase, INR: International Normalized Ratio of prothrombin time, A/G ratio: Albumin/Globulins ratio, a Significant difference from normal volunteers at $p<0.05$, b Significant difference from 0 week value at $p<0.05$

Table 2: Liver function tests in serum of hepatitis C patients of Child-B class before treatment and at different observation periods after DDB therapy

Item	Normal volunteers (15)	Weeks post DDB treatment 0 (6)	2 (6)	6 (2)
APC (ml/min/kg)	0.42	0.23 a	0.26	0.19
	± 0.03	±0.03	±0.03	±0.06
ALT (U mL^{-1})	19.53	57.83 a	29.67 b	36.00a
	±2.53	±9.32	±7.19	±1.00
AST (U mL^{-1})	29.67	90.17 a	71.83	93.50
	±2.63	±14.76	±14.46	±34.50
GGT (U L^{-1})	21.13	58.83 a	77.83	104.50
	±1.80	±15.73	±14.92	±44.50
ALP (U L^{-1})	130.80	132.33	154.83	188.50
	±10.9	±31.46	±22.48	±56.50
Bilirubin (mg dL^{-1})	0.81	2.22 a	1.77	2.45
	±0.06	±0.41	±0.73	±1.05
INR	1.21	2.20 a	1.96	1.89
	±0.03	±0.37	±0.42	±0.03
Total proteins (g dL^{-1})	7.60	6.47 a	6.48	6.75
	± 0.13	±0.55	±0.26	±0.55
Albumin (g dL^{-1})	4.17	3.22 a	2.75	2.30
	±0.13	±0.36	±0.43	±0.40
Globulins (g dL^{-1})	3.43	3.27	3.75	4.45
	± 0.13	±0.67	±0.54	±0.15
A/G ratio	1.28	1.37	0.93	0.50
	± 0.08	±0.46	±0.34	±0.10

Values are means ± SEM, ( ) Number of patients or volunteers, APC: antipyrine clearance, ALT: Alanine aminotransferase AST: Aspartate aminotransferase, GGT: Gamma Glutamyl Transferase, ALP: Alkaline Phosphatase, INR: International Normalized Ratio of prothrombin time, A/G ratio: Albumin/Globulins ratio, a Significant difference from normal volunteers at $p<0.05$. b Significant difference from 0 week value at $p<0.05$

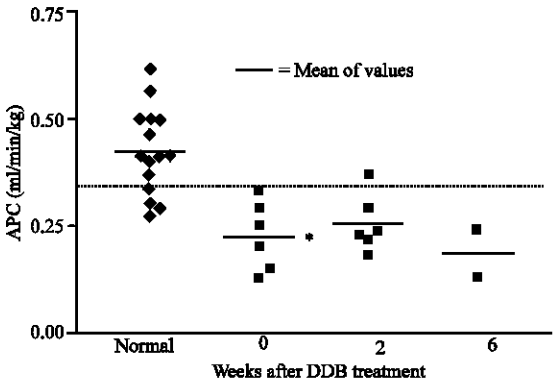

Fig. 2: Antipyrine clearance in normal volunteers and Child-Pugh class B HCV patients before and at different observation periods post DDB treatment

After DDB treatment no significant improvement in APC (Table 2 and Fig. 2) was recorded 2 and 6 weeks after treatment.

Seven Child-C patients complied to follow-up treatment protocol, but only two patients completed 6 weeks follow-up after treatment. Before treatment and compared to normal volunteers, a significant ($p<0.001$) less APC was recorded. Two and 6 weeks after DDB treatment insignificant improvement in APC was recorded when values were compared to basal values (Table 3 and Fig. 3).

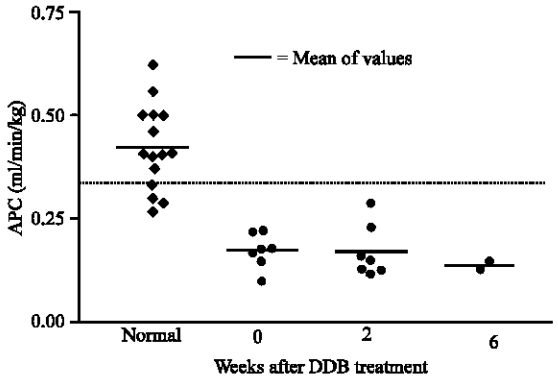

Fig. 3: Antipyrine clearance in normal volunteers and Child-Pugh class C HCV patients before and at different observation periods post DDB treatment

Table 3: Liver function tests in serum of hepatitis C patients of Child-C class before treatment and at different observation periods after DDB therapy

		Weeks post DDB treatment		
Item	Normal volunteers (15)	0 (7)	2 (7)	6 (2)
APC (ml/min/kg)	0.42 ± 0.03	0.17 a ±0.02	0.17 ±0.02	0.14 ±0.01
ALT (U mL^{-1})	19.53 ±2.53	51.14 a ±8.13	35.29 ±6.43	21.50 ±6.50
AST (U mL^{-1})	29.67 ±2.63	57.57 a ±15.96	64.29 ±15.07	57.50 ±6.50
GGT (U L^{-1})	21.13 ±1.80	48.43 a ±7.29	53.57 ±9.68	30.00 ±8.00
ALP (U L^{-1})	130.80 ±10.9	145.71 ±15.31	148.00 ±13.13	118.50 ±6.50
Bilirubin (mg dL^{-1})	0.81 ±0.06	4.27 a ±1.18	2.73 ±0.75	4.15 ±1.65
INR	1.21 ±0.03	3.22 a ±0.55	3.14 ±0.68	4.96 ±2.54
Total proteins (g dL^{-1})	7.60 ± 0.13	6.43 a ±0.34	6.91 ±0.30	7.45 ±0.35
Albumin (g dL^{-1})	4.17 ±0.13	2.30 a ±0.17	2.73 ±0.18	2.80 ±0.20
Globulins (g dL^{-1})	3.43 ± 0.13	4.13 a ±0.24	4.19 ±0.33	4.65 ±0.55
A/G ratio	1.28 ± 0.08	0.54 a ±0.04	0.67 ±0.10	0.60 ±0.10

Values are means ± SEM, () Number of patients or volunteers, APC: antipyrine clearance, ALT: Alanine aminotransferase AST: Aspartate aminotransferase, GGT: Gamma Glutamyl Transferase, ALP: Alkaline Phosphatase, INR: International Normalized Ratio of prothrombin time, A/G ratio: Albumin/Globulins ratio, a Significant difference from normal volunteers at $p<0.05$

DISCUSSION

Increase of serum levels of Aminotransferases (ALT and AST) reported as sensitive measure of hepatocellular membrane damage and leakage (Dufour *et al*., 2000), as they are considered to be good reflectors of disease activity in chronic viral hepatitis (Wojcicki *et al*., 2002; Giannini *et al*., 2005). However, aminotransferase levels are known to fluctuate between abnormal increased to normal levels in some HCV patients (NIH, 1998). Clinically, DDB was reported to markedly improve the symptoms and impaired liver functions, such as bilirubin, alpha-fetoprotein, elevated serum ALT in HBV patients (Liu, 1987) and reported to normalize serum ALT in most of chronic liver patients (Liu, 1989; Huber *et al*., 2004). Experimentally, some authors reported that DDB reduced ALT levels that were elevated due to hepatic damage induced by Ccl4, D-galactosamine, prednisolone (Liu *et al*., 1979, 1982), thioacetamide (Yu *et al*., 1987) and tamoxifen (El-Beshbishy, 2005). Several mechanisms of DDB effect on ALT level were suggested such as anti-lipid peroxidation action (Liu *et al*., 1979, 1982) antioxidant action (El-Sawy *et al*., 2002), anticytolysis action (Mak and Ko, 1997), anabolic action (Liu, 1989) and metabolic induction of cytochrome P450 enzymes (Kim *et al*., 1995). However, it was suggested that DDB may not be genuinely improving liver function, but only influencing the synthesis and/or degradation of ALT in the hepatocytes by an unknown mechanism (Huber *et al*., 2004).

In this study, the only significant change after DDB administration was the decrease in ALT level, which was significantly increased than normal together with AST in all Child-Pugh classes of patients before treatment. While, parameters expressing severity of hepatitis (INR, total bilirubin and albumin) and GGT were not improved. This data was in agreement with Huber *et al*. (2004) who denied any improvement in the elevated GGT levels and Salama *et al*. (2004) who denied any improvement in the elevated GGT and serum bilirubin levels after DDB treatment. But Liu (1989) and Montasser (2001) observed a significant reduction in serum bilirubin. In this study, the reduction in the total protein and A/G ratio (in Child-B and C patients) and the elevation in globulin (in Child-C patients) were not improved after DDB treatment, although Liu (1989) reported a significant improvement in A/G ratio 3 months after treatment.

In the present study, Child-A class of patients treated with DDB did not show any significant improvement in APC. Contrary to our findings, Zhu *et al*. (1999) reported increase in APC after treating rats intoxicated with CCl4 with *Schisandra chinensis* fruit, which contains both schisandrin B and C. This contradictory may be due to the difference in the model of liver injury, host, type of treatment and/or method of APC estimation (thin layer chromatography).

APC can be considered a sensitive hepatic functional assay than conventional liver function tests

(Jorquera *et al*., 2001) and it showed not only quantitative changes between hepatic patients and normal volunteers, but also showed qualitative changes between the different Child-Pugh classes (A, B and C) of patients before treatment (Mahmoud *et al*., 2007). Moreover, APC was significantly less in Child-A patients examined in this study before treatment, while most of the biochemical parameters were within normal ranges. On the other hand, Podalsky and Isselbacher (1998) reported that changes of prothrombin time can be considered the earliest indicator of the liver insult in severe liver injury and Giannini *et al*. (2005) recommended monitoring of prothrombin time in order to assess the risk of acute liver failure. In our study, also INR of prothrombin time, that negatively correlated with APC (Mahmoud *et al*., 2007) was not improved in patients after DDB treatment.

CONCLUSION

Although findings on the response Child-B and C classes to DDB should be taken carefully because of the limited number of patients complying to follow-up periods, yet results of Child-A class of patients can be considered confidently. Therefore, it can be concluded that dimethyl diphenyl bicarboxylate did not improve the metabolic or biochemical liver function that was impaired due to hepatitis C virus infection.

REFERENCES

Bartholomew, R. and A. Delany, 1964. Proc. Australian Assoc. Clin. Biochem., 1: 64.

Deutsche Gesellschaft für Klinische Chemie [DGKC], 1972. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie. Recommendation of German Society of Clinical Chemistry (GSCC). J. Clin. Chem. Clin. Biochem., 10: 182-193.

Dufour, D.R., J.A. Lott, F.S. Nolte, D.R. Gretch, R.S. Koff and L.B. Seeff, 2000. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin. Chem. 46: 2027-2049.

Echnizen, H., Y. Ohta, H. Shirataki, K. Tsukamoto, N. Umeda, T. Oda and T Ishizaki, 1990. Effects of subchronic treatment with natural human interferons on antipyrine clearance and liver function in patients with chronic hepatitis. J. Clin. Pharmacol., 30: 562-567.

El-Beshbishy, H.A., 2005. The effect of Dimethyl Dimethoxy Biphenyl dicarboxylate (DDB) against Tamoxifen-induced Liver Injury in Rats: DDB use is curative or protective. J. Bioch. Mole. Biol., 38: 300-306.

El-Sawy, S.A., A.M. El-Shafey and H.A. El-Bahrawy, 2002. Effect of dimethyl diphenyl bicarboxylate on normal and chemically-injured liver. East Mediterr. Health J., 8: 95-104.

Giannini, E.G., R. Testa, V. Savarino, 2005. Liver enzyme alteration: A guide for clinicians. Can. Med. Assoc. J., 172: 367-379.

Gornall, A.G., C.J. Bardawil and M.M. David, 1949. Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem., 177: 751-766.

Hougie, C., 1982. The biochemistry of blood coagulation. In Triplett DA Laboratory evaluation of coagulation. Am. Soc. Clin. Pathol. Press, Chicago, pp: 2.

Huber, R., B. Hockenjos and H.E. Blum, 2004. DDB treatment of patients with chronic hepatitis. Hepatol., 39: 1732-1733.

Jendrassik, L. and P. Grof., 1938. Vereinfachte photometrische Methoden zur Bestimmung des Bilirubins. Biochem., 297: 81-89.

Jorquera, F., M. Almar, A. Linares, J.L. Olcoz, L. Rodrigo and J. Gonzalez-Gallego, 2001. Antipyrine clearance and metabolite formation in primary biliary cirrhosis. Dig. Dis. Sci., 46: 352-359.

Kim, S.G., S.Y. Nam, H.C. Chung, S.Y. Hong and K.H. Jung, 1995. Enhanced effectiveness of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate in combination with garlic oil against experimental hepatic injury in rats and mice. J. Pharm. Pharmacol., 47: 678-682.

Lee, K.H., 2000. Research and future trends in the pharmaceutical development of medicinal herbs from Chinese medicine. Public Health Nutr., 3: 515-522.

Liu, G.T., 1987. Therapeutic effects of Biphenyl Dimethyl Dicarboxylate (DDB) on chronic viral hepatitis B. Proc. Chin. Acad Med. Sci. Peking Union Med. Coll., 2: 228-233.

Liu, G.T., 1989. Pharmacological actions and clinical use of Fructus Schizandrae. Chin. Med. J., 102: 740-749.

Liu, G.T., G.F. Wang, H.L. Wei, T.T. Bao and Z.Y. Song 1979. A comparison of the protective actions of biphenyl dimethyl-dicarboxylate, trans-stilbene, alcoholic extracts of Fructus schizandrae and Ganoderma against experimental liver injury in mice. Acta. Pharm. Sin., 14: 598-604.

Liu, G.T., H.L. Wei and Z.Y. Song, 1982. Further studies on the protective action of Biphenyl Dimethyl-Dicarboxylate (BDD) against experimental liver injury in mice. Yao Xue Xue Bao., 17: 101-106.

Mahmoud, M., R. Abdel-Kader, M. Hasanin, S. Saleh and S. Botros, 2007. Antipyrine clearance in comparison to conventional liver function tests in hepatitis C virus patients. European Jouran of Pharmacology (in Press).

Mak, D.H. and K.M. Ko, 1997. Alterations in susceptibility to carbon tetrachloride toxicity and hepatic antioxidant/detoxification system in streptozotocin-induced short-term diabetic rats: Effects of insulin and Schisandrin B treatment. Mol. Cell. Biochem., 175: 225-232.

Marques, M.P., O.M. Takayanagui and V.L. Lanchote, 2002. Albendazole metabolism in patients with neurocysticercosis: Antipyrine as a multifunctional marker drug of cytochrome P450. Braz. J. Med. Biol. Res., 35: 261-269.

McHutchison, J.G., S.C. Gordon, E.R. Schiff, L. Mitchell, W.M. Shiffman, V.K. Lee, Z.D. Rustgi, M.H. Goodman, S. Ling and J.K. Cort, 1998. Interferon alpha-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N. Engl. J. Med., 339: 1485-1492.

Mehta, M.U., R. Venkataramanan, G.J. Burckart, R.J. Ptachcinski, S.L. Yang, J.A. Gray, D.H. Van Thiel and T.E. Starzl, 1986. Antipyrine kinetics in liver disease and liver transplantation. Clin. Pharmacol. Ther., 39: 372-377.

Montasser, M.F., 2001. Clinical and laboratory studies of DDB in treatment of chronic hepatitis C: 4-Combination therapy with amantadine hydrochloride, a pilot study in Egypt. Ain. Shams. Med. J., 52: 125-142.

National Institute of Health (NIH), 1998. Consensus Development Conference Panel statement: Management of hepatitis C. National Institutes of Health, Bethesda, Maryland. Hepatol., 26: 2-10.

Persijn, J.P. and W. Van der Slik, 1976. A new method for the determination of gamma-glutamyl transferase in serum. J. Clin. Chem. Clin. Biochem., 14: 421-427.

Peyton, B.G., T.L. Spears, A. Lindsey, J. Lindsey, V.K. Sharma and J. Raufman, 1999. A survey of the use of herbal medicine in patients with hepatitis C. Hepatol., 30: 191A.

Podalsky, D.K. and K.J. Isselbacher, 1998. Derangement of hepatic metabolism. Principles of internal medicine. McGraw Hill, New York, pp: 1667-1672.

Poulsen, H.E., S. Loft, 1988. Antipyrine as a model drug to study hepatic drug-metabolizing capacity. J. Hepatol., 6: 374-382.

Pugh, R.N., I.M. Murray-Lyon, J.L. Dawson, M.C. Pietroni and R. Williams, 1973. Transection of the oesophagus for bleeding oesophageal varices. Br. J. Surg., 60: 646-649.

Rasi, G., D. DiVirgilio, M.G. Mutchnick, F. Collela, P. Sinibaldi-Vallebona, P. Pierimarchi, B. Valli and E. Garaci, 1996. Combination thymosin alpha-1 and lymphoblastoid interferon treatment in chronic hepatitis C. Gut., 39: 679-683.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Salama, H.M., A.R. Amer, O.M. Hammad and W.F. El-Sayed, 2004. Effect of DDB monotherapy and in combination with amantadine hydrochloride and ribavirin in patients with chronic hepatitis C virus infection. Sci. Med. J. ESCME., 16: 1.

Shen, D.G., M. Araki, I. Higuchi, K. Matsumoto, M. Tamai, K.T. Liu and H. Sugita, 1987. The effect of DDB on dystrophic hamsters an *in vivo* and *in vitro* study. Muscle Nerve, 10: 391-396.

St Peter, J.V. and W.M. Awni, 1991. Quantifying hepatic function in the presence of liver disease with phenazone (antipyrine) and its metabolites. Clin. Pharmacokinet, 20: 50-65.

Sturgill, M.G. and G.H. Lambert, 1997. Xenobiotic-induced hepatotoxicity: mechanisms of liver injury and methods of monitoring hepatic function. Clin. Chem. 43: 1512-1526.

Sun, H. and G.T. Liu, 2005. Chemopreventive effect of dimethyl dicarboxylate biphenyl on malignant transformation of WB-F344 rat liver epithelial cells. Acta. Pharmacol. Sin., 26: 1339-1344.

Teunissen, M.W., J.E. Meerburg-van der Torren, N.P. Vermeulen and D.D. Breimer, 1983. Automated high-performance liquid chromatographic determination of antipyrine and its main metabolites in plasma, saliva and urine, including 4,4'-dihydroxyantipyrine. J. Chromatogr., 278: 367-378.

Wojcicki, J., K. Kozlowski, M. Drozdzik and M. Wojcicki, 2002. Comparison of MEGX (monoethylglycinexylidide) and antipyrine tests in patients with liver cirrhosis. Eur. J. Drug. Meta. Pharmacokinet, 27: 243-247.

Yu, H.Q., X.Y. Yang, Y.X. Zang and J.Z. Shi, 1987. Biphenyl dimethyl dicarboxylate in treating and preventing hepatitis due to drug poisoning. Chin. Med. J., 100: 122-123.

Zeuzem, S., S.V. Feinman, J. Rasenack, E.J. Heathcote, M.Y. Lai, E. Gane, J. O'Grady, J. Reichen, M. Diago, A. Lin, J. Hoffman and M.J. Brunda 2000. Peginterferon alfa-2a in patients with chronic hepatitis C.N. Engl. J. Med., 343: 1666-1672.

Zhu, M., K.F. Lin, R.Y. Yeung and R.C. Li, 1999. Evolution of the protective effects of Schisandra chinensis on phase I drug metabolism using a CCl4 intoxication model. J. Ethnopharmacol., 67: 61-68.