Effect of dimethyl diphenyl bicarboxylate on normal and chemically-injured liver

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تأثير ديميثيل ديفينيل البيكاربوكسيلات على الكبد السوي والكبد المتخرب بالمواد الكيميائية

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الخلاصة: تقيّم هذه الدراسة تأثير هذه المادة على الكبد السوي والكبد المتخرب بالمواد الكيميائية، إذ لم يُلاحظ أي تأثير لها على الإنزيمات الكبدية بعد إعطائها للفئران السوية، أما لدى إعطائها للفئران التي تعاني أكبادها من أذيّة كيميائية المنشأ فإن مستويات الإنزيمات المرتفعة تنخفض بشكل واضح، كما تزداد بوضوح المستويات المنخفضة لكلٍ من الغلوتاثيون وبيروكسيدار الغلوتاثيون ومختزلة الغلوتاثيون مع انخفاض شديد في المالون ديالدهيد ونازعة هيدروجين الغلوكوز – 6 – فوسفات في كلٍ من الأكباد السوية والمتأذية بالمواد الكيميائية. وقد بيّنت الفحوصات النسيجية الباثولوجية تحسّنا طفيفا تلو المعالجة بهذه المادة. ويمكن الاستنتاج أن هذه المواد تأثيرات مفيدة على الإنزيمات الكبدية وأن لها خواص هامة مضادة للأكسدة في كلٍ من الأكباد السوية والمتأذية بالمواد الكيميائية، وقد تكون مفيدة سريريًا في معالجة التهاب الكبد الفيروسي "بي" لدى الإنسان.

ABSTRACT This study evaluates the effect of DDB on normal and chemically-injured liver. When given to normal rats DDB had no significant effect on liver enzymes, but in chemically-injured rats there was a significant decrease in the elevated levels of liver enzymes. DDB produced a significant increase in reduced glutathione, glutathione peroxidase and glutathione reductase, and a significant decrease in malondialdehyde and glucose 6 phosphate dehydrogenase in both normal and chemically-injured liver. The histopathology examinations showed a slight improvement with DDB administration. DDB has a beneficial effect on liver enzymes and possesses significant antioxidant properties in normal and chemically-injured liver, and may therefore be clinically useful in treating chronic viral hepatitis B in humans.

Effet du diphénylbicarboxylate de diméthyle sur un foie normal et sur un foie ayant subi une atteinte

ABSTRACT Cette étude évalue l'effet du diphénylbicarboxylate de diméthyle (DDB) sur un foie normal et sur un foie ayant subi une atteinte chimique. Administré à des rats normaux, le DDB n'avait pas d'effet significatif sur les enzymes hépatiques, mais chez des rats ayant eu une atteinte chimique, il y avait une diminution significative des taux élevés d'enzymes hépatiques. Le DDB a produit une augmentation significative du glutathion réduit, de la glutathion-peroxydase et de la glutathion-réductase, ainsi qu'une diminution significative du malondialdéhyde et de la glucose-6-phosphate déshydrogénase ainsi bien dans le foie normal que dans un foie ayant subi une atteinte chimique. Les examens histopathologiques ont montré une légère amélioration avec l'administration de DDB. Le DDB a un effet bénéfique sur les enzymes hépatiques et possède d'importantes propriétés antioxydantes pour un foie normal et pour un foie ayant subi une atteinte chimique ; il peut donc être utile au plan clinique pour traiter l'hépatite virale B chronique chez l'homme.

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Introduction

Treatment options for common liver diseases such as cirrhosis, fatty liver, chronic hepatitis are problematic. All too often the treatment is worse than the disease [1]. Dimethyl diphenyl bicarboxylate (DDB) is a synthetic analogue of schizandrin C, one of the components isolated from fructus schizandrae [2]. DDB is widely used by Chinese herbalists to treat impaired liver function [3].

Many researchers have examined the hepatotonic and hepatoprotective effect of DDB; for example, Ip and colleagues reported that the protective effect of Schizandra was more evident after induction of liver damage with carbon tetrachloride (CCl_4) [4]. Similarly Mak and Ko suggested that DDB had a hepatoprotective effect on CCl_4-induced liver toxicity [5]. However, Kim and colleagues investigated the effect of DDB and observed that either single or repeated DDB pretreatment did not alter the hepatotoxicity induced by CCl_4 [6].

In China DDB has been tested clinically since 1979 on patients with viral hepatitis B. The results indicate that DDB markedly improves impaired liver functions, such as elevated serum glutamic pyruvic transaminase, bilirubin and α-fetoprotein, and also improves the symptoms of the patients [7]. As the absorption rate of DDB tablets from the gastrointestinal tract is only about 30%, a new preparation of DDB (pilules) with higher bioavailability was created in 1982 [8], and was investigated.

While all these published reports exa mined the hepatoprotective effect of DDB, its curative effectiveness against chemically-induced hepatic injury and/or its effect on normal liver have not been studied. The present study was therefore designed to assess the clinical use of DBB by evaluating its effect and mechanism of action on normal and chemically-injured liver.

Methods

Eighty male albino rats (weight range 200 250g) were caged with a free supply of food and water. After acclimatization, they were randomly assigned into 8 groups of 10 rats each to study:

- The effect of DDB on normal rats. Three experimental groups of 10 rats were each given DDB intragastrically in a dose of 50, 150 or 300 mg per kg body weight once daily for 21 consecutive days. A group of 10 normal control rats received 1 mL/kg of saline.

- The effect of DDB on CCl_4-treated rats. All rats were injected intrape-ritoneally with a 50% solution of CCl_4 in olive oil at 1 mL/kg body weight twice weekly for 1 week. Three experi-mental groups of 10 rats each received DDB as described above. A group of 10 control rats received CCl_4 only, as described before.

All animals were killed, and blood and liver samples were obtained. The following serum enzymes were measured:

- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the Reitman and Frankel method using the bioMérieux kit [9]. All methods for determination of serum aminotransferases depend on their activity and so the results are not expressed as concentrations. The colorimetric determination of ALT and AST depends on determining the amounts of oxalacetate and pyruvate formed from the 2,4-dinitrophenyl-hydrazine of oxalacetic and pyruvic acid, the colour of which can be read at 520 nm.

- Lactate dehydrogenase (LDH) was measured by monitoring the increasing intensity of NADH production as a result of oxidation of lactate to pyruvate [10].
- Sorbitol dehydrogenase (SDH) activity was determined by measuring NADH formed due to oxidation of sorbitol to fructose [11].
- Malondialdehyde (MDA) is a thiobarbituric acid reactant substance. It is considered as the end product of lipid peroxidation with thiobarbituric acid to form a pink coloured product that is read at 530 nm [12].

Part of the excised liver was fixed in 10% formalin for 48 hours then transferred to 70% ethyl alcohol, processed and embedded in paraffin blocks. Sections of 5 μm thickness were stained with haematoxylin and eosin (H&E) for routine histopathology examination. The other part of the liver was used to prepare a liver homogenate by homogenizing 1 g of wet liver tissue in 10 mL ice-cold buffer solution.

The following parameters were measured in the liver homogenate.

- Glutathione peroxidase (GPX) activity was determined according to the method described by Flohe and Gunzler which is based on the oxidation of reduced glutathione (GSH) by GPX in the presence of cumene, glutathione reductase and NADPH. One unit of GPX activity was equal to mmol of NADPH oxidized per minute per mg protein [13].
- Glutathione reductase (GRD) activity was determined using the method described by Carlberg and Mannervik, which is based on aliquots of the tissue homogenate added to NADPH-GSSG buffered solution pH 7.0 and measured at 340 nm. One unit of GRD activity

was equal to nmol of NADPH oxidized per minute per mg protein [14].

- Hepatic glucose-6-phosphate dehydrogenase (G6PDH) was determined by the method of Glock and McClean, which is based on the conversion of G6P by G6PDH into 6-phosphoglucuronic acid in the presence of NADP with concomitant formation of NADPH [15].
- GSH levels were determined fluorimetrically according to the method of Hissin and Hilf using O-phthaldehyde and phosphate-EDTA buffer at pH 8.0. The fluorescence was measured at 420 nm with excitation at 350 nm [16].

Statistical analyses were performed using unpaired *t*-tests [17].

Results

Table 1 shows that in doses of 50, 150 or 300 mg/kg of body weight DDB did not produce any significant change in the serum enzymes levels of ALT, AST, LDH and SDH compared with saline control rats. However, at doses of 150 and 300 mg/kg it caused a significant increase in the concentration of GSH, GPX and GRD enzyme activities and a significant reduction in G6PDH and MDA.

Table 2 shows the effects on CCl_4 pretreated rats. It is clear that CCl_4 alone caused a significant increase in all serum enzymes measured and a significant reduction in hepatic GSH, GPX and GRD compared with saline control rats. In addition, the MDA concentration and G6PDH activity were significantly increased compared with the controls.

When given to CCl_4-treated rats in a dose of 50 mg/kg, DDB had no significant effect on any parameters studied except for

Table 1 **Effect on rats of oral administration of dimethyl diphenyl bicarboxylate (DDB) for 21**

Group	ALT (U/L)	AST (U/L)	LDH (U/L)	SDH (mU/mg)
Control ($n = 10$)	21.30 ± 2.49	70.10 ± 4.53	211.80 ± 5.71	19.60 ± 4.92
DDB (50mg/kg) ($n = 10$)	20.20 ± 4.54	69.70 ± 9.18	207.00 ± 9.17	25.80 ± 11.60
t_1	0.67	0.12	1.4	1.55
DDB (150mg/kg) ($n = 10$)	19.00 ± 3.02	66.80 ± 7.64	208.00 ± 6.25	21.20 ± 4.59
t_2	1.85	1.17	1.42	0.75
DDB (300mg/kg) ($n = 10$)	19.50 ± 3.81	66.60 ± 4.80	208.70 ± 18.90	19.90 ± 2.33
t_3	1.25	1.67	0.49	0.17

Values are given as mean values ± standard deviation.
t_2 *for DDB 150 mg/kg versus saline control group.*
*Statistically significant at P < 0.05.
AST = aspartate aminotransferase.
SDH = sorbitol dehydrogenase.
GPX = glutathione peroxidase.
G6PDH = glucose-6-phosphate dehydrogesanse.

Table 2 **Effect on rats of oral administration of dimethyl diphenyl bicarboxylate (DDB) for 21**

Group	ALT (U/L)	AST (U/L)	LDH (U/L)	SDH (mU/mg)
Control ($n = 10$)	21.30 ± 2.49	70.10 ± 4.53	211.80 ± 5.71	19.60 ± 4.92
CCl_4 alone ($n = 10$)	139.50 ± 26.77	306.40 ± 24.77	458.60 ± 58.46	499.40 ± 43.50
t_1	13.89*	29.65*	13.28*	34.64*
CCl_4 + DDB (50 mg/kg body weight) ($n = 10$)	101.40 ± 16.66	200.80 ± 17.75	407.20 ± 65.29	493.70 ± 23.96
t_2	3.83*	10.95*	1.85	0.36
CCl_4 + DDB (150 mg/kg body weight) ($n = 10$)	47.70 ± 10.07	104.80 ± 14.30	319.00 ± 17.26	395.50 ± 40.96
t_3	10.07*	12.30*	7.24*	5.49*
CCl_4 + DDB (300 mg/kg body weight) ($n = 10$)	21.60 ± 13.84	140.50 ± 4.23	278.00 ± 6.59	311.00 ± 31.95
t_4	12.36*	20.87*	9.69*	11.03*

Values are given as mean values ± standard deviation.
t_2 *for DDB 50 mg/kg versus CCl_4 alone.*
t_4 *for DDB 300 mg/kg versus CCl_4 alone.*
ALT = alanine aminotransferase.
LDH = lactate dehydrogenase.
MDA = malondialdehyde.
GRD = glutathione reductase.
GSH = reduced glutathione.

consecutive days on liver function and on hepatic antioxidant status

MDA (nmol/g)	GPX (mU/mg)	GRD (mU/mg)	G6PDH (mU/mg)	GSH (nmol/mg)
56.70 ± 4.85	67.60 ± 4.85	6.80 ± 3.25	1.14 ± 0.29	8.90 ± 3.35
50.30 ± 10.70	70.20 ± 4.94	8.70 ± 2.90	0.87 ± 0.33	10.34 ± 0.76
1.75	1.19	1.38	1.93	1.32
28.10 ± 5.09	80.60 ± 3.03	10.20 ± 3.12	0.60 ± 0.27	13.17 ± 0.38
12.88*	7.18*	2.38*	4.50*	3.99*
24.90 ± 4.68	84.80 ± 4.85	12.30 ± 2.21	0.49 ± 0.23	13.78 ± 0.27
14.93*	7.93*	4.44*	5.42*	4.60*

t_i for DDB 50 mg/kg versus saline control group.
t_j for DDB 300 mg/kg versus saline control group.
ALT = alanine aminotransferase.
LDH = lactate dehydrogenase.
MDA = malondialdehyde.
GRD = glutathione reductase.
GSH = reduced glutathione.

consecutive days on liver function and on hepatic antioxidant status after CCl₄ treatment

MDA (nmol/g)	GPX (mU/mg)	GRD (mU/mg)	G6PDH (mU/mg)	GSH (nmol/mg)
56.70 ± 4.85	67.60 ± 4.85	6.00 ± 3.25	1.14 ± 0.29	8.90 ± 3.35
123.20 ± 17.80	22.30 ± 12.08	3.75 ± 0.72	1.42 ± 0.16	3.26 ± 0.89
11.39*	10.99*	2.90*	2.80*	5.17*
110.00 ± 11.64	23.50 ± 3.87	3.55 ± 0.59	1.34 ± 0.14	4.32 ± 1.72
1.96	0.29	0.69	1.14	1.73
84.70 ± 7.48	33.80 ± 2.96	5.40 ± 0.62	1.21 ± 0.04	7.00 ± 0.67
6.30*	2.93*	5.50*	4.15*	10.69*
72.10 ± 5.07	37.60 ± 3.83	6.05 ± 2.14	1.17 ± 0.01	8.16 ± 0.61
8.74*	3.83*	3.19*	5.00*	14.41*

t^i for CCl4 versus saline control group.
t_j for DDB 150 mg/kg versus CCl₄ alone.
*Statistically significant at $P < 0.05$.
AST = aspartate aminotransferase.
SDH = sorbitol dehydrogenase.
GPX = glutathione peroxidase.
G6PDH = glucose-6-phosphate dehydrogesanse.

a significant decrease in the elevated levels of ALT and AST compared to CCl₄ alone (Table 2). When given in a dose of 150 or 300 mg/kg, however, DDB caused a significant decrease in the elevated levels of all serum enzymes (ALT, AST, LDH and SDH) compared with CCl₄ alone (Table 2). At the two higher doses, DDB also caused a significant increase in the depressed GSH levels and GPX and GRD activities compared with CCl₄ alone. It also caused a significant reduction in the elevated MDA concentration and G6PDH activity compared with CCl₄-treated rats.

Comparing liver sections of DDB-treated rats with the control group showed no significant pathological changes with DDB (Figure 1). Histopathology study of liver sections in animals treated with CCl₄, however, showed extensive dilatation of central veins and blood sinusoids associated with diffuse extensive vacuolar degeneration of the liver in seven out of 10 animals (Figure 2). The remaining three animals showed moderate dilatation of the liver vascular bed with moderate vacuolar degeneration of liver cells mainly in the centrilobular zone. Using DDB as a treatment for CCl₄ intoxication it was found that there was marked resolution of hepatocellular toxicity, manifested histopathologically in the liver sections as minimal fatty change with dilated sinusoid and hypertrophied Kupffer cells (Figure 3).

Discussion

This study has shown that DDB has a beneficial effect on hepatotoxicity induced by CCl₄ in rats.

When CCl₄ was given to rats it caused a marked liver cell injury as assessed by a significant increase in all the serum enzymes studied (ALT, AST, LDH and SDH)
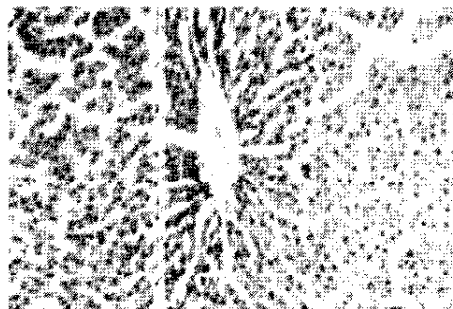

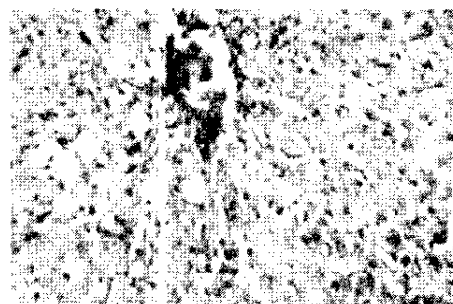Figure 1 **Section of normal liver tissue (H&E×125)**

Figure 2 **Section from liver tissue after CCl₄ intoxication showing zonal necrosis plus extensive diffuse vacuolar degeneration of the liver cells (fatty tissue shows dilatation and congestion of central vein with central change) (H&E×250)**

and by histopathological changes. When DDB was administered to CCl₄ hepatotoxic rats it caused a significant reversal in these elevated levels of hepatic enzymes (ALT, AST, LDH). In normal control rats DDB had no effect on the levels of serum enzymes studied. This decrease in liver enzymes could be an index of reduction of hepatic and extrahepatic tissue damage. At the higher doses, DDB also caused a
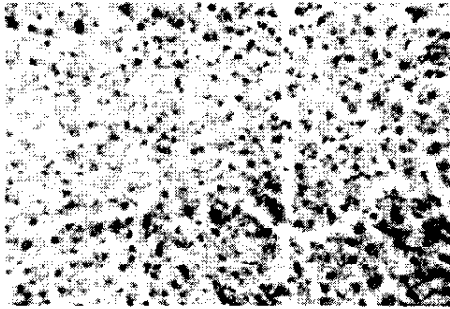
Figure 3 **Section from liver tissue after dimethyl diphenyl bicarboxylateadministration following CCl₄ intoxication showing minimal fatty changes with dilated sinusoids (H&E×125)**

significant decrease in the elevated SDH levels, denoting a reduction of liver cytolysis caused by CCl₄. This indicates that DDB causes a near nor-malizing of liver enzyme levels after chemically-induced injury and a partial improvement of the clinical chemistry parameters.

However, administration of DDB produced only a minimal effect on the histopathology lesions that follow chemical damage of the liver by CCl₄. This could be explained by the promotion of certain aspects of anabolic metabolism by DDB, which has been reported to increase serum protein biosynthesis and glycogenesis [18]. This may lead to partial repair of injured liver cells.

With regard to the oxidant defence system, it is clear that when given to saline control rats DDB causes a significant increase in the activities of hepatic enzymes GSH, GPX and GRD, and a significant reduction in G6PDH and MDA. These results accord with those of Fu and Liu, who reported that DDB is beneficial to normal hepatocyte activity [19]. DDB has been shown to have antioxidant activity

against oxygen free radicals [20]. These highly reactive short-lived molecules are generated during ordinary metabolism and can damage the cell membrane. DDB as an antioxidant prevents the formation of these free radicals, because the increased GSH levels observed after DDB administration act as a first defence against oxidants by maintaining the cellular integrity [21].

DDB also stimulates the activities of GSH-related enzymes, such as GPX and GRD. The increases in glutathione levels acts as substrate for GPX and GRD enzymes that clear the toxic intermediates formed in the cell.

Another possible explanation for the effect of DDB in saline control rats is that DDB acts as non-enzymatic antioxidant by enhancing hepatic ascorbic acid and maintaining the levels of vitamin E [4].

CCl₄ injected to rats greatly activates the intensity of free radical formation and lipid peroxidation, as evidenced by an increase in MDA. It also causes damage to the endoplasmic reticulum, as indicated by a significant increase in G6PDH levels and it impairs the antioxidant system by inhibiting GPX and GRD activities and decreasing the GSH level. The level of depletion of GSH has been used as an index of oxidative stress [22], and a sign that hepatic cells are utilizing more antioxidant defences [23].

The higher doses of DDB caused a significant enhancement of the antioxidant defence system in CCl₄-treated rats, as shown by increased GSH, GPX and GRD activities, and decreased MDA and G6PDH. The mechanisms by which DDB improves the function of damaged liver are not completely understood, but several possibilities can be suggested.

First, schizandrin C has been shown to inhibit lipid peroxidation (MDA formation) despite the absence of pro-oxidant activity [24]. The improvement afforded by DDB

might be attributed to the enhancement of antioxidants possibly through increasing the activities of GPX and GRD.

Another possible mechanism for the partial curative effect of DDB is the protective effect it provides against glutathione depletion caused by CCl_4. Ip and colleagues have suggested that DDB enhances the hepatic mitochondrial glutathione redox status as well as increasing the mitochondrial GRD activity in both normal and CCl_4-treated mice [25]. The ability of DDB to sustain hepatic mitochondrial GSH levels may represent the antioxidant action of this drug. Sagara and colleagues have suggested that the elevation of intracellular level of GSH by DDB is an adaptive response to oxidative stress [26].

DDB could be involved in maintaining a variety of intracellular functions including detoxification. Intracellular detoxification induces an action on microsomal cytochromes P450 of endoplasmic reticulum and P450 plays a key role in the detoxification mechanism of the liver [27]. This

could be another explanation for the partial curative effect of DDB.

Another mechanism for the effect of DDB is increasing the hepatic levels of vitamins C or E. which would explain the non-enzymatic antioxidant effect of this drug. Ip and colleagues have suggested that DDB is able to increase the hepatic levels of vitamin C and vitamin E in mice intoxicated with CCl_4 [20].

We can conclude that, although DDB does not appear to bring about a complete reversal of drug-induced injury in the liver and has only minimal effects on histopathological lesions, it partially improves the liver enzyme levels and also acts as an antioxidant and antilipid peroxidant, enhancing detoxification and protecting against glutathione depletion. This improvement of impaired liver function suggests that DDB could be used for the treatment of chronic viral hepatitis B in humans as it has been shown to reduce the main symptoms of patients and its side-effects are rare and not serious.

References

1. Luper S. A review of plants used in the treatment of liver disease: part 1. *Alternative medicine review*, 1998, 3(6):410–21.

2. Shen DC et al. The effect of DDB on dystrophic hamsters an *in vivo* and *in vitro* study. *Muscle and nerve*, 1987, 10(5):391–6.

3. Liu ZY, Cui O, Liu GT. Effect of dimethyl 4-4 dimethoxy-5,6,5,6-dimethylene dioxybiphenyl-2,2-dicarboxylate (DDB) on several phenotypes of Bel-7402 hepatocarcinoma cell line and its mechanism. *Acta pharmaceutica sinica*, 1996, 31(2): 101–16.

4. Ip SP et al. Schisandrin B protects against carbon tetrachloride toxicity by enhancing the mitochondrial glutathione redox status in mouse liver. *Free radical biology and medicine*, 1996, 21(5):709–12.

5. Mak DH, Ko KM. Alterations in susceptibility to carbon tetrachloride and hepatic antioxidant detoxification system in streptozotocin-induced short-term diabetic rats: effect of insulin and schisandrin B treatment. *Molecular and cellular biochemistry*, 1997, 175(1–2): 225–32.

6. Kim SN et al. Effect of DDB on chemical induced liver injury. *Biological and pharmaceutical bulletin*, 1999, 22(1):93–5.

7. Liu KT et al. A comparison of protective actions of diphyenyl-dimethyl-dicarboxylate, trans-stilbene alcoholic extracts of Fructus schizandrae and Ganoderma against experimental liver injury in mice. Acta pharmaceutica sinica, 1979, 14(10):598-4.

8. Liu GT, Wei HL, Song ZY Further studies on the protective action of diphenyl dimethyl bicarboxylate (DDB) against experimental liver injury in mice. Acta pharmaceutica sinica, 1982, 17(2):101-5

9. Reitman S, Frankel S. Colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases activity. American journal of clinical pathology, 1957, 28:56-63.

10. Wacker WEC, Ulmer DD, Vallee BL. Metalloenzymes and myocardial infarction. New England journal of medicine, 1956, 225:449-3.

11. Dooley J. Sorbitol dehydrogenase and its use in toxicology testing in laboratory animals. Laboratory animal. 1984. 20-1.

12. Trotta RJ, Sullivan SG, Stern SD. Lipid peroxidation and hemoglobin degradation in red blood cells exposed to t-butyl hydroperoxide. Effects of the hexose monophosphate shunt as mediated by glutathione and ascorbate. Biochemical journal, 1982, 204(2):405-15.

13. Flohe L, Gunzler WA. Assay of glutathione peroxidase. Methods in enzymology, 1984, 105:114-21.

14. Carlberg I Mannervik B. Glutathione reductase. Methods in enzymology, 1985, 113:484-90.

15. Glock GE, McClean P. Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. Biochemical journal, 1953, 55:440-5.

16. Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Analytical biochemistry, 1976, 74:214-26.

17. Cuzmo JW. Basic statistics for the health sciences. California, Mayfield Publishing Company, 1984:18.

18. Liu GT. Pharmacological actions and clinical use of fructus schizandrae. London clinical medical journal, 1989, 102(10):740-9.

19. Fu T, Liu G. Protective effects of dimethyl-4-4-dimethoxy-5, 6, 5, 6-dimethylene dioxybiphenyl-2,2-dicarboxylate on damages of isolated rat hepatocyte induced by carbon tetrachloride and D-galactosamine. Biomedical and environmental science, 1992, 5(3):185-94.

20. Ip SP, Ko KM. The crucial antioxidant action of schisandrin B in protecting against carbon tetrachloride hepatotoxicity in mice: A comparative study with butylated hydroxytoluene. Biochemical pharmacology, 1996, 52(1):1687 93.

21. Gohil K et al. Blood glutathione oxidation during human exercise. Journal of applied physiology, 1988, 64:115-7.

22. Pascoe GA, Read DJ. Cell calcium, vitamin E, and the thiol redox systen in cytotoxicity. Free radical biology and medicine, 1989, 6:209-24.

23. Ip SP et al. Effect of schisandrin B on hepatic glutathione antioxidant system in mice: protection against carbon tetrachloride toxicity. Planta medica, 1995, 61(5):398-401.

24. Mak DH et al. Effects of schisandrin B and alpha-tocopherol on lipid peroxidation, in vitro and in vivo. Molecular and cellular biochemistry, 1996, 165(2):161-5.

25. Ip S, Yiu HY, Kos KM. Differential effect of schisandrin B and dimethyl diphenyl bicarboxylate (DDB) on hepatic mitchondrial glutathione redox status in carbon tetrachloride intoxicated mice. *Molecular and cellular biochemistry*, 2000, 205(1–2):111–4.

26. Sagara Y et al. Cellular mechanisms of resistance to chronic oxidative stress.

Free radical biology and medicine, 1998, 24(9):1375–89.

27. Wong ГW, Chan WY, Lee ST. Resistance to carbon tetrachloride induced hepatotoxicity in mice, which lack CYP2EI expression. *Toxicology and applied pharmacology*, 1998, 153:109–18.

Note from the Editor

We would like to draw our readers' attention to the subject and author indexes for Volume 6 (2000) which are distributed as an insert in the present issue.