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

S.A. El-Sawy\textsuperscript{1}, A.M. El-Shafey\textsuperscript{2} and H.A. El-Bahrawy\textsuperscript{3}

ABSTRACT This study evaluated 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 diphenylbicarboxylate de diméthyle sur un foie normal et sur un foie ayant subi une atteinte

ABSTRACT Cette étude évalue l'effet du diphenylbicarboxylate 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 glutathione-peroxydase et de la glutathione-reductase, 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 anti-oxydantes 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 dicarboxylate (DDB) is a synthetic analogue of schizandrin C, one of the components isolated from Tructus Schizandraceae [2]. DDB is widely used by Chinese herbalists to treat impaired liver function [3].

Many researchers have examined the hepatoprotective effect of DDB; for example, Iw and colleagues reported that the protective effect of Schizandra was more evident after induction of liver damage with carbon tetrachloride (CCL₄) [4]. Similarly Mak and Ko suggested that DDB had a hepatoprotective effect on CCl₄-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 CCl₄ [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 (pills) with higher bioavailability was created in 1982 [8], and was investigated.

While all these published reports examined 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 DDB 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 CCl₄-treated rats.** All rats were injected intra-peritoneally with a 50% solution of CCl₄ in olive oil at 1 mL/kg body weight twice weekly for 1 week. Three experimental groups of 10 rats each received DDB as described above. A group of 10 control rats received CCl₄ 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 oxalurate and pyruvate formed from the 2,4-dinitrophenyl-hydrazone 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 Flora 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 mmol 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-phosphogluconic 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 470 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 CCl₄ pretreated rats. It is clear that CCl₄ 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 CCl₄-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

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>LDH (U/L)</th>
<th>SDH (mU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>21.30 ± 2.49</td>
<td>70.10 ± 4.53</td>
<td>211.80 ± 5.71</td>
<td>19.60 ± 4.92</td>
</tr>
<tr>
<td>DDB (50mg/kg) (n = 10)</td>
<td>20.20 ± 4.54</td>
<td>69.70 ± 9.18</td>
<td>207.00 ± 9.17</td>
<td>25.80 ± 11.60</td>
</tr>
<tr>
<td>t₀</td>
<td>0.67</td>
<td>0.12</td>
<td>1.4</td>
<td>1.55</td>
</tr>
<tr>
<td>DDB (150mg/kg) (n = 10)</td>
<td>19.00 ± 3.02</td>
<td>66.80 ± 7.64</td>
<td>208.00 ± 6.25</td>
<td>21.20 ± 4.59</td>
</tr>
<tr>
<td>t₂</td>
<td>1.85</td>
<td>1.17</td>
<td>1.42</td>
<td>0.75</td>
</tr>
<tr>
<td>DDB (300mg/kg) (n = 10)</td>
<td>19.50 ± 3.81</td>
<td>66.60 ± 4.80</td>
<td>208.70 ± 18.90</td>
<td>19.90 ± 2.33</td>
</tr>
<tr>
<td>t₄</td>
<td>1.25</td>
<td>1.67</td>
<td>0.49</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Values are given as mean values ± standard deviation.
*Statistically significant at P < 0.05.

AST = aspartate aminotransferase.
SOD = superoxide dismutase.
GPX = glutathione peroxidase.
G5PDH = glucose-6-phosphate dehydrogenase.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>LDH (U/L)</th>
<th>SDH (mU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>21.30 ± 2.49</td>
<td>70.10 ± 4.53</td>
<td>211.80 ± 5.71</td>
<td>19.60 ± 4.92</td>
</tr>
<tr>
<td>CCl₄ alone (n = 10)</td>
<td>139.50 ± 26.77</td>
<td>306.40 ± 24.77</td>
<td>458.60 ± 58.46</td>
<td>499.40 ± 43.50</td>
</tr>
<tr>
<td>t₁</td>
<td>13.89*</td>
<td>29.65*</td>
<td>13.28*</td>
<td>34.64*</td>
</tr>
<tr>
<td>CCl₄ + DDB (50 mg/kg body weight) (n = 10)</td>
<td>101.40 ± 16.66</td>
<td>200.80 ± 17.75</td>
<td>307.20 ± 65.29</td>
<td>403.70 ± 23.96</td>
</tr>
<tr>
<td>t₂</td>
<td>3.83*</td>
<td>10.95*</td>
<td>1.85</td>
<td>0.36</td>
</tr>
<tr>
<td>CCl₄ + DDB (150 mg/kg body weight) (n = 10)</td>
<td>47.70 ± 10.07</td>
<td>104.00 ± 14.30</td>
<td>310.00 ± 17.26</td>
<td>306.60 ± 40.96</td>
</tr>
<tr>
<td>t₃</td>
<td>10.07*</td>
<td>12.30*</td>
<td>7.24*</td>
<td>5.49*</td>
</tr>
<tr>
<td>CCl₄ + DDB (300 mg/kg body weight) (n = 10)</td>
<td>21.60 ± 13.84</td>
<td>140.50 ± 4.23</td>
<td>270.00 ± 0.59</td>
<td>311.00 ± 31.96</td>
</tr>
<tr>
<td>t₄</td>
<td>12.36*</td>
<td>20.87*</td>
<td>9.69*</td>
<td>11.03*</td>
</tr>
</tbody>
</table>

Values are given as mean values ± standard deviation.
*Statistically significant at P < 0.05.

ALT = alanine aminotransferase.
LDH = lactate dehydrogenase.
MDA = malondialdehyde.
GRD = glutathione reductase.
GSH = reduced glutathione.
### Consecutive Days on Liver Function and on Hepatic Antioxidant Status

<table>
<thead>
<tr>
<th>MDA (nmol/g)</th>
<th>GPX (mU/mg)</th>
<th>GRD (mU/mg)</th>
<th>G6PDH (mU/mg)</th>
<th>GSH (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.70 ± 4.85</td>
<td>67.60 ± 4.85</td>
<td>6.80 ± 3.25</td>
<td>1.14 ± 0.29</td>
<td>8.90 ± 3.35</td>
</tr>
<tr>
<td>50.30 ± 10.70</td>
<td>70.20 ± 4.94</td>
<td>8.70 ± 2.90</td>
<td>0.87 ± 0.33</td>
<td>10.34 ± 0.76</td>
</tr>
<tr>
<td>1.75</td>
<td>1.19</td>
<td>1.38</td>
<td>1.93</td>
<td>1.32</td>
</tr>
<tr>
<td>28.10 ± 5.09</td>
<td>80.60 ± 3.03</td>
<td>10.20 ± 3.12</td>
<td>0.60 ± 0.27</td>
<td>13.17 ± 0.38</td>
</tr>
<tr>
<td>12.50*</td>
<td>7.10*</td>
<td>2.00*</td>
<td>4.00*</td>
<td>3.99*</td>
</tr>
<tr>
<td>24.90 ± 4.68</td>
<td>84.80 ± 4.85</td>
<td>12.30 ± 2.21</td>
<td>0.49 ± 0.23</td>
<td>13.78 ± 0.27</td>
</tr>
<tr>
<td>14.93*</td>
<td>7.93*</td>
<td>4.44*</td>
<td>5.42*</td>
<td>4.60*</td>
</tr>
</tbody>
</table>

*ti, for DDB 50 mg/kg versus saline control group.
*t2, 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

<table>
<thead>
<tr>
<th>MDA (nmol/g)</th>
<th>GPX (mU/mg)</th>
<th>GRD (mU/mg)</th>
<th>G6PDH (mU/mg)</th>
<th>GSH (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.70 ± 4.05</td>
<td>67.60 ± 4.05</td>
<td>0.60 ± 0.23</td>
<td>1.14 ± 0.29</td>
<td>8.90 ± 3.35</td>
</tr>
<tr>
<td>123.20 ± 17.80</td>
<td>22.30 ± 12.08</td>
<td>3.75 ± 0.72</td>
<td>1.42 ± 0.16</td>
<td>3.26 ± 0.89</td>
</tr>
<tr>
<td>11.39*</td>
<td>10.99*</td>
<td>2.90*</td>
<td>2.80*</td>
<td>5.17*</td>
</tr>
<tr>
<td>110.00 ± 11.64</td>
<td>23.50 ± 3.87</td>
<td>3.55 ± 0.59</td>
<td>1.34 ± 0.14</td>
<td>4.32 ± 1.72</td>
</tr>
<tr>
<td>1.96</td>
<td>0.29</td>
<td>0.69</td>
<td>1.14</td>
<td>1.73</td>
</tr>
<tr>
<td>84.70 ± 7.48</td>
<td>33.80 ± 2.96</td>
<td>5.40 ± 0.62</td>
<td>1.21 ± 0.04</td>
<td>7.00 ± 0.67</td>
</tr>
<tr>
<td>6.30*</td>
<td>2.93*</td>
<td>5.50*</td>
<td>4.15*</td>
<td>10.69*</td>
</tr>
<tr>
<td>72.10 ± 5.07</td>
<td>37.60 ± 3.83</td>
<td>6.05 ± 2.14</td>
<td>1.17 ± 0.01</td>
<td>8.16 ± 0.61</td>
</tr>
<tr>
<td>8.74*</td>
<td>3.83*</td>
<td>3.19*</td>
<td>5.00*</td>
<td>14.41*</td>
</tr>
</tbody>
</table>

*ti, for CCI4 versus saline control group.
*t2, 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 dehydrogenase.
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 vascular 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 vascular 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) and by histopathological changes. When DDB was administered to CCl₄ hepatotoxic rats it caused a significant reversal in those 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
significant decrease in the elevated SDH levels, denoting a reduction of liver cytology caused by CCl₄. This indicates that DDB causes a near normalization 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 CCl₄. 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 CCl₄-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 CCl₄ [27].

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


