Cytogenetic and biochemical studies on the effect of DDB in albino mice and their embryos.

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Abstract

Introduction: DDB (Dimethyl – 4,4’ – dimethoxy – 5,6,5’,6’ – dimethene – dioxybiphenyl – 2,2 – dicarboxylate) is important drug of medicine not expensive since large number of people are using it in virus B and C cases for very large periods extend to many years. The protective of DDB on chemically induced damage was studied in primary cultures of mammals hepatocytes.

Results: This work study of cytogenetic and biochemical effect of DDB, in mice using the chromosomes of bone marrow of male and pregnant female shown some changes with liver embryos. Also germ cells of testes given non significant aberration when compared with control.

As well as some biochemical parameters in serum and tissues, shown non significant changes in nucleic acid, total protein, total cholesterol, total glucose, total triglycerides and lactate dehydrogenase (LDH). Also, enzyme analysis of liver function and kidney.

Introduction

Investigation of DDB (Dimethyl dimethoxy biphenyl Dicarboxylate) is synthetic analogue of schisandrin C which is a traditional Chinese medicine since 1977 and was tried in treatment of chronic HCV in china and Egypt with encouraging results (Montasser 2000 and 2001).

DDB with the chemical structure given below has been used for the treatment of viral hepatitis and drug – induced liver injury in china for about ten years. Liver represents the largest organ in the mammalian body. DDB had been shown to be able to protect the liver against hepatotoxins such as CCL2, and thiaoacetamide to induce liver microsomal cytochrome P-450 in mice and rats (Liu et al 1997, 1982 and Liu and Lesca 1982).

This drug was also shown to inhibit the mutagenic action of benzo pyrene (BP) and aflatoxine (AFB1) in Ames test (Liu and Lesca 1982 & Wang 1984) and to inhibit (AFB1) – induced hepatocarcinogenesis in rats (Yan et al, 1986).

DDB which is a synthetic analogue to schisandrin C (active ingredient in schisandra chinesis extract) showed the powerful hepatoprotective and antiviral activity (Gao et al, 2005). It was frequently used in Egypt in the management of chronic viral and non-viral hepatitis. It showed reduction of hepatocellular carcinoma thought the decrease of alpha-fetoprotein levels (Montaser, 1999).

Treatment options for common liver disease such as cirrhosis, fatty liver chronic hepatitis. In china DDB has been tested clinically science Liu (1979) on patients with viral hepatitis B. The results indicate that DDB markedly improve impaired liver function. Similarly Mak and Ko (1997) Suggested that DDB had hepatoprotective effect on CCL4 – induced liver toxicity.

However Kim and Colleagues (1999) investigated the effect of DDB and observed that either single or repeated DDB pretreatment did not alter hepatotoxicity induced by CCL4.

This work aims to study the cytogenetic and biochemical effects of DDB (Bifendate pilutes) in mice using the chromosomes of bone marrow cells, liver embryos and germ cells.

Some biochemical parameters like Nucleic acid protein and enzymes were analyzed.
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Material and Methods

**Chemicals:**
**Definition of drug:** Trade name: DDB
Generic name: Bifendate Pilules

**Chemical name and structural formula:**
Chemical name: Dimethyl - 4,4' - dimethoxy - 5,6,5',6' - dimethylene dioxy biphenyl - 2,2 dicarboxylate

Molecular formula: $C_{20}H_{18}O_{10}$
Molecular weight: 418.36

*Structural formula:*

```
O
O
C=O

O
O
C=O

O
O
C=O

O
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Category: Antihepatitis agent

**Kits:** Glutamic – pyruvic transaminase GPT (ALT)
Glutamic – oxaloacetate transaminase GOT (AST)
Kit, Total protein, Cholesterol, glucose, triglycerides
Kit, Creatinina and Urea Kit (C cromastes linear chemicals S.T.)

Statistical analysis were performed using un paired t-tests (Sokall and Rohlf 1969).

**Animals:**
80 adult fertile male and adult virgin female Swiss albino mice 8-12 weeks old weight between 25-30 g were used from the Department of Animal House colony of National Research Center.

All animals used in this study is divided into two main part:

**First part:** Cytogenetic part

**Second part:** Biochemical analysis

Experiment were carried out to evaluate the effect of DDB drug using different cytogenetic study and Biochemical parameters.

Animals were divided into two main equal group:

**Group I:** 20 animals (10 male and 10 pregnant female mice) which considered untreated mice or normal standard were gives distilled water for 30 days.

**Group II:** 20 animals (10 male and 10 pregnant female mice were fed orally by DDB at 0.75 mg/kg/day for 30 days.

After the last dose animals were scarified.

**A. Cytogenetic Part:**
Chromosomes preparation for 40 male and pregnant female mice were caged individually and were randomly divided into two groups:

**Group I:** 10 male and 10 pregnant female mice served as control and were administrated with (0.25 ml) distilled water.

**Group II:** 10 male and 10 pregnant female mice were orally administrated with (0.75 mg/kg/day) DDB for 30 day. (with 5 embryos for each mother).

In each mice (male and pregnant female mice) study somatic chromosomal aberration was made for bone marrow (Yosida et al, 1971) with liver lived embryos of mother treated DDB and control (50 embryo each group) according to (Romagnano et al., 1985).

In each male study germ cells (Spermatocytes) according to (Evans et al., 1964).

**B. Biochemical Part:**
For determined biochemical parameters for 40 male and pregnant female mice with their embryos. 40 animals were used in form of two groups as same as cytogenetic part.

The blood of mice (male and pregnant female mice) was collected and serum was separated to determine serum glucose (Trinder, 1969); Triglycerides (Fassati and Prencipe, 1982); Cholesterol (Richmond, 1973); Liver enzyme GPT (ALT) and GOT (AST) according to Reitman and Frankel (1975); Kidney enzyme Creatinine (Bartles et al, 1972) and Urea (Fawcett and Soctt., 1960).

Nucleic acid and total protein were determined in different tissues (Liver, Kidney, Testes and Liver embryos). DNA (Peares, 1985). RNA (Schneider 1957) and Total protein according to (Peter, 1968).
Results

I- Cytogenetic effect of DDB in mice:

1. Effect on males

The cytogenetic effect of male shown in table (1, 2). The main types of structural chromosomal aberration are gaps, breaks, centromeric attenuations (C.A) and endomitosis in somatic cell of bone morrow. Table (1) showed that DDB (0.75 mg /kg / b.w) did not produce any significant change of chromosomal aberration than control in somatic cell. Table (2) shown the effect of DDB in the same male mice after 30 days in germ cells (spermatocytes) (x-y univalent, Autosmal univalent and chain). It is clear that DDB alone did not caused significant change when compared with control.

2. Effect on pregnant females:

In pregnant mice given DDB (0.75 mg/ kg/ b.w) daily for 30 days (Table 3) the result indicate non significant aberration on somatic cells (structural and numerical) when compared with untreated mice.

Table (4) shown the changes of structural chromosomal aberration in liver embryo of treated mother with DDB (0.75 mg/kg/b.w) when compared with control embryo there is non significant changes.

Mitotic index in all tables where a non significant difference appeared with treated mice (DDB) than control mice.

II- Biochemical effect of DDB in mice:


The results were absorbed the effect of DDB on DNA, RNA and protein in different tissue of male mice (Table 5). There were some different but this different was non significant than control in all parameters.

As same as in pregnant female and liver embryos the effect of DDB on DNA, RNA and protein presented in table (6). The changes in all parameters gives non significant change when compared with control mother and embryo.

2. Effect of DDB in Enzymatic serum mice:

On the other hand, from male and pregnant female mice that exposure to DDB daily oral (0.75 mg/kg/d.w) for 30 days results of Enymatic serum (glucose, Triglycerides, cholesterol and LDH) (Table 7) did not give any changes than untreated mice which considered standard value.

3. Effect of DDB in liver function of mice:

In this result we studied the effect of DDB drug in tissues and serum of mice by determined the value liver enzymes GOT (AST) and GPT (ALT) (Table 8, 9).

The result indicate there were decrease in the value of tissues (liver male, liver pregnant female an liver embryo) and serum (male and pregnant female) than control mice.

4. Effect of DDB in Kidney function of mice:

Table (10) indicate the serum of male and pregnant female mice which treated with DDB give nonsignificant data in creatinine and urea when compared with control.
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Table (1): Effect of DDB on chromosomes of bone marrow (somatic cells) in male mice after 30 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of mice</th>
<th>No of Cells scored</th>
<th>Structural Aberration</th>
<th>Numerical aberration</th>
<th>Mitotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Break</td>
<td>End mitosis</td>
<td>C. A</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>500</td>
<td>0.4%</td>
<td>0.4%</td>
<td>1%</td>
</tr>
<tr>
<td>DDB dray</td>
<td>10</td>
<td>500</td>
<td>0.4%</td>
<td>0.4%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Table (2): Effect of DDB on chromosomes of testes (Spermatocytes) in male mice after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of examined male mice</th>
<th>No of cells scored</th>
<th>Structural Aberration</th>
<th>Total %</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>X-Y univalent</td>
<td>Autosomal univalent</td>
<td>Chain</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>500</td>
<td>0.6%</td>
<td>0.8%</td>
<td>--</td>
</tr>
<tr>
<td>DDB dray</td>
<td>10</td>
<td>500</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

Table (3): Effect of DDB on chromosomes of bone marrow in pregnant female mice after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of mice</th>
<th>No of Cells scored</th>
<th>Structural Aberration</th>
<th>Numerical aberration</th>
<th>Mitotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Break</td>
<td>End mitosis</td>
<td>C. A</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>500</td>
<td>0.6%</td>
<td>1.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>DDB dray</td>
<td>10</td>
<td>500</td>
<td>1%</td>
<td>1.4%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

Table (4): Effect of DDB on chromosomes mice liver embryo of treated mother after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of mice</th>
<th>No of Cells scored</th>
<th>Structural Aberration</th>
<th>Numerical aberration</th>
<th>Mitotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Break</td>
<td>End mitosis</td>
<td>C. A</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>2500</td>
<td>0.36%</td>
<td>0.2%</td>
<td>0.6%</td>
</tr>
<tr>
<td>DDB dray</td>
<td>50</td>
<td>2500</td>
<td>0.52%</td>
<td>0.24%</td>
<td>0.8%</td>
</tr>
</tbody>
</table>
Table (5): Effect of DDB on DNA, RNA and protein in male mice after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Testes</td>
<td>Liver</td>
<td>Kidney</td>
<td>Testes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DNA mg/g</td>
<td>0.418 ± 0.005</td>
<td>0.303 ± 0.064</td>
<td>0.312 ± 0.023</td>
<td>0.426 ± 0.005</td>
<td>0.309 ± 0.01</td>
<td>0.323 ± 0.005</td>
<td></td>
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</tr>
<tr>
<td>Total RNA mg/g</td>
<td>0.271 ± 0.033</td>
<td>0.178 ± 0.006</td>
<td>0.190 ± 0.021</td>
<td>0.276 ± 0.035</td>
<td>0.186 ± 0.021</td>
<td>0.193 ± 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein g/g</td>
<td>7.041 ± 0.161</td>
<td>4.955 ± 0.183</td>
<td>4.436 ± 0.137</td>
<td>7.126 ± 0.208</td>
<td>4.959 ± 0.118</td>
<td>4.527 ± 0.143</td>
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</tr>
</tbody>
</table>

Table (6): Effect of DDB on DNA, RNA and protein in pregnant female and their embryos after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DNA mg/g</td>
<td>0.369 ± 0.006</td>
<td>0.252 ± 0.009</td>
<td>0.208 ± 0.004</td>
<td>0.378 ± 0.005</td>
<td>0.282 ± 0.006</td>
<td>0.219 ± 0.004</td>
<td></td>
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</tr>
<tr>
<td>Total RNA mg/g</td>
<td>0.239 ± 0.012</td>
<td>0.156 ± 0.011</td>
<td>0.137 ± 0.008</td>
<td>0.245 ± 0.014</td>
<td>0.158 ± 0.008</td>
<td>0.147 ± 0.014</td>
<td></td>
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</tr>
<tr>
<td>Total Protein g/g</td>
<td>7.027 ± 0.188</td>
<td>4.579 ± 0.166</td>
<td>3.052 ± 0.196</td>
<td>7.111 ± 0.134</td>
<td>4.581 ± 0.161</td>
<td>3.112 ± 0.174</td>
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</tr>
</tbody>
</table>

Table (7): Effect of DDB on biochemical markers in male and pregnant female mice after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male Control</th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg/dl</td>
<td>89.121 ± 0.586</td>
<td>89.118 ± 0.629</td>
<td>79.83 ± 0.583</td>
<td>79.662 ± 0.587</td>
<td></td>
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</tr>
<tr>
<td>Triglycerides mg/dl</td>
<td>66.339 ± 1.412</td>
<td>66.024 ± 1.534</td>
<td>50.8 ± 4.269</td>
<td>49.6 ± 2.782</td>
<td></td>
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</tr>
<tr>
<td>Cholesterol mg/dl</td>
<td>193.836 ± 1.556</td>
<td>193.64 ± 1.583</td>
<td>184.328 ± 1.564</td>
<td>184.192 ± 1.639</td>
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</tr>
<tr>
<td>LDH U/L</td>
<td>259.692 ± 5.004</td>
<td>258.723 ± 5.707</td>
<td>250.033 ± 5.651</td>
<td>247.095 ± 5.107</td>
<td></td>
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</tbody>
</table>

Table (8): Effect of DDB on liver enzymes in tissues male and pregnant female with their embryos after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GOT(AST) U/L</td>
<td>52.4 ± 2.234</td>
<td>45.6 ± 1.459</td>
<td>39.5 ± 1.687</td>
<td>50.4 ± 3.159</td>
<td>43.7 ± 1.853</td>
<td>39 ± 1.956</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPT (ALT) U/L</td>
<td>58.8 ± 1.96</td>
<td>56.1 ± 2.241</td>
<td>43.8 ± 0.991</td>
<td>25.53 ± 1.251</td>
<td>52.53 ± 1.343</td>
<td>43 ± 0.861</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (9): Effect of DDB on liver enzymes male and pregnant female in serum after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GOT(AST) U/L</td>
<td>65 ± 2.222</td>
<td>63.4 ± 2.924</td>
<td>62. ± 1.757</td>
<td>61.3 ± 1.112</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPT(ALT) U/L</td>
<td>67.55 ± 1.565</td>
<td>66.2 ± 2.232</td>
<td>64.9 ± 2.616</td>
<td>63 ± 2.841</td>
<td></td>
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</tr>
</tbody>
</table>
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Table (10): Effect of DDB on kidney function male and pregnant female in serum after 30 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Pregnant female</td>
</tr>
<tr>
<td>Control</td>
<td>0.896 ± 0.016</td>
<td>0.788 ± 0.018</td>
</tr>
<tr>
<td>DDB</td>
<td>0.843 ± 0.023</td>
<td>0.733 ± 0.021</td>
</tr>
</tbody>
</table>

Discussion

The present study showed the effect of DDB drug on mice (male, Pregnant female and their embryos). In order to investigated the role of DDB in mammals because HCV infection is wide spread problem following mainly blood transfusion, surgical procedures, operations and dental procedure (Donaldson et al., 1994).

Lenord (2005) shown that the DDB is a beneficial effect on mammal. The changes of chromosomal mice (male, Pregnant female and their embryos) non significant which mean safty and no mutagenic action was detected.

IP et al. (2000) added that the treating mice with DDB daily oral dose did not produce any significant alteration in plasma alanine amino transferase (ALT) and sorbital dehydrogenase (SDH) activity. Also, Lui et al. (2005) and Goa et al. (2005) supported that the reduced elevated on ALT and AST on serum and Tissues after treated with DDB drug. Adding to Lui (1989) which said that the DDB improved the liver function in female rats as the elevated serum, GPT and GOT in liver hepatities patients have been decreased.

In carcinogen-induce DNA damage that the DDB is able to directly or indirectly protective effect (Ging and Liue, 1992) and Gao et al. (2005). Also, Chang et al. (2004) and Gao et al. (2005) added that the DDB protect the inhibition of RNA which agreement with our results.

Fu and Liu (1992) reported that when normal rats were given DDB daily for 10 days, the free ribosomal protein and RNA liver increased significantly. Also, liver glycogen and blood glucose was reduced with DDB.

Mowafy (2004) investigated that the DDB when given befor meal to patients with chronic hepatitis C which is big problem in our country. The ALT and AST is lowing in serum and no effects on blood urea and serum creatinine.

The protective action of the drug mainly referred to its corrective action on protein synthesis with repair of the structure and function of damaged hepatocytes (Xa et al 1997).

This study concluded that the DDB effective observed no side effect (Salame et al. 2004) which equal to our results. Also, DDB caused a significant reduction in elevated levels of all serum enzymes (ALT, AST, LDH and SDH) compared with levels of CCL4.

This improvement of impair liver function suggests that the DDB could be used for treatment of chronic viral hepatitis B in human as it has been to reduce the main symptoms of patients and its side effect are rare and not serious (El Saway et al. 2002).

In conclusion, the present data indicate that oral administration of DDB, have a beneficial effects on damaged liver cells to prevent lipid peroxidation and improve antioxidant enzyme activiles.

The data obtained from study revealed that DDB commediate its biochemical effects to protect action against liver.

The toxicity of DDB is very rare No teratogenic or mutagenic action was detected No untoward effects of DDB have been observed. DDB is not expensive with no side effects.

References


24. Peter T (1968): proposals for standardization of total protein colorimetric
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دراسة التأثير الوراثي الخلوي والكيميائي الحيوي لعقار ال د د بً (DDB)
على الجرزان البيضاء الصغيرة وأجنتها

عادل الركيب - ** أميرة عبذ الرؤوف

* استاذ بكلية الطب جامعة الأزهر (فرع بنين) القاهرة
** المركز القومي للبحوث فسم بيولوجيا الخلية - الدقي – القاهرة

نظراً لانتشار فيروس سي وبي بالكبد وله انتشرت أدوية كثيرة لعلاجه ولأنها غالبية الثمن ولها تأثير فلقد قمنا بدراسة عقار ال د د بً (DDB) وتأثيره على الثدييات لأنه غير مكلف ويستخدم لفترة طويلة. ولقد قمنا بالدراسة على جزيج وراثة خلوي وجزء كيميائي حيوي.

الجزء الأول الدراسة الخلوي قمنا بدراسة كرموسومات الفئران البيضاء الصغيرة لكل من الذكور والإناث الحوامل في نخاع العظم ووجد أنه لا يوجد تأثير بالمقارنة للمجموعة الضابطة الغير معاملة وأيضاً دراسة كرموسومات الكبد بالنسبة لأغذية الإناث الحوامل المعاملة بال (DDB) وجدت أنها لم تتأثر تأثير معنوي.

والنسبة للذكور قمنا بدراسة كرموسومات الخصية ووجدت أيضاً أنها لم تتأثر بالمقارنة بالمجموعة الغير معاملة.

الجزء الثاني قمنا بدراسة التأثير الكيميائي الحيوي للعقار (DDB) على خلايا بعض الأعضاء (الكبد والكلي والخصيتين وكبد الأجنحة) والدم ووجد أنه لا يوجد تغير في الحمض النووي (دن أ رن أ) والبروتين والكلسترول والجلوكوز والترقى لجسرين والكتات دهيدروجينز وعائي أنزيمات الكبد ولا الكلى من هنا وجد أن عقار (DDB) ليس له تأثير ضار ورخيص الثمن وممكن استعماله بأمان.