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Full Length Research Article

Diphenyl Dimethyl Bicarbonylate as an Effective Treatment for Chemical-Induced Fatty Liver in Rats

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ABSTRACT

To assess the protective effect of diphenyl dimethyl bicarbonylate (DDB) in carbon tetrachloride (CCl₄) – induced fatty liver in male rats .Fatty liver was induced by single intraperitoneal (IP) CCl₄ dose of 0.2mL/kg , treated group was given the same CCl₄ dose , then given oral daily doses of DDB as 300 mg /kg body weight for 4 weeks . A control group , given saline orally was run against the other two groups . Each group consisted of 10 male Wistar albino rats. Liver weight (in g) , liver tissue contents of total protein (TP) , total lipids (TL) , phospholipids (PL) , total cholesterol (TC) and triglycerides (TG) as mg / g wet tissue were investigated. CCl₄ treatment significantly increased liver tissue weight , TL , TC , TG , but decreased both TP and PL . DDB treatment after CCl₄ intoxication , recovered these variables near normalcy. The study substantiates the protective action of DDB against CCl₄ – induced fatty liver , through assessment of most tissue parameters known to be disrupted in fatty liver . The drug significantly decreased both liver weight , TL , TC , TG and increased both PL and TP , so improved fat mobilization from chemical-induced fatty liver (Afr. J. Biomed. Res. 9:77 – 81, May 2006)

Keywords: Fatty liver , rats , carbon tetrachloride , DDB , total protein , total lipids , total cholesterol , phospholipids , triglycerides

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INTRODUCTION

The liver represents the largest organ in the vertebrate body . It is the main site of most metabolic reactions . Being the main gate of the body ; it is affected by drugs , exhibiting different degrees of toxicity (Venukumar and Latha , 2002) . Liver biopsy is still the gold standard for assessment of fatty liver , although abdominal sonographic examination proved reproduce able results in clinical practice for safety , ethical and screening purposes (Steinmaurer *et al* , 1984) .

Fatty changes in the liver are closely associated with metabolic impairments such as hyperglycemia and dyslipidemia (Angelico *et al* , 2003) . Liver cirrhosis is always accompanied by increased levels of triglycerides and cholesterol , this was attributed to decreased hepatic lipase activity and low lecithin : cholesterol acyl transferase activity (Iglesias *et al* , 1996) . The high prevalence of fatty liver may be attributed to increased intake of fat (Ganmaa *et al* , 2003) , in association with increased intestinal permeation (De Meo *et al* , 2002) .

Diphenyl Dimethyl Bicarboxylate (DDB), which is a synthetic analogue to Schisandrin C (active ingredient in Schisandra chinensis extract), showed a 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 promising results in control of liver cirrhosis and reduction of hepatocellular carcinoma through the decrease of alpha fetoprotein levels (Montaser ,1999) . The protective action of the drug was mainly referred to its corrective action on protein synthesis and the repair of structure and function of damaged hepatocytes (Xu *et al* , 1997) . In combination with garlic oil; DDB showed an additional advantage in lowering plasma lipids (Park *et al* , 2005) .Till now ; trials made to assess the role of DDB in the treatment of fatty liver are scarce . The present work is designed to study the role of the drug in the treatment of carbon tetrachloride – induced fatty liver in rats through the evaluation of most variables included in this trouble on the liver tissue level

MATERIALS AND METHODS

Chemicals

DDB was obtained from the Egyptian market, imported from Guang Zhou Xing Qun Pharmaceutical Company (China) , in the form of dried pillules , commonly known in Egypt as Yellow Pill . It was ground as powder form , then suspended in water using gum acacia just prior to administration.CCl₄ was obtained from Prolabo , UK. All other chemicals were of reagent grade .

Animals and diets

Thirty male Wistar albino rats weighing 100 – 120 g were purchased from Faculty of Veterinary Medicine , Zagazig University , Egypt . The animals were housed in polypropylene cages under controlled temperature and light cycle (12 hours light and 12 hours dark) . Water was supplied ad libitum . All rats were fed fixed diet consisted of dried wheat , barley , skimmed dry milk , carrots and kept for one week before medication to accommodate . Animals were classified into three groups (12 rats each) , the first served as control and was given 0.2 mL / kg body weight (BW) saline as a single IP dose , the second group constituted hepatointoxicated rats , was given pure CCl₄ , 0.2 mL / kg BW, as single IP dose (Kim *et al* , 1999) .The third group was the drug – treated animals , given CCl₄ as a single dose , then given DDB suspension (300 mg / kg .B .W, intragastrically ,IG) , daily for 4 weeks (Fu and Liu ,1992) . Animals were kept starved 10 hours before being decapitated , liver tissues were immediately dissected out , blotted off blood between filter papers , washed by saline and then weighed .Then , tissues were instantaneously frozen in liquid nitrogen right biochemical investigations .

Biochemical assays

For determination of liver tissue content of lipids , 200 mg wet tissue was homogenized with 10 mL of chloroform / methanol (2 : 1 , V / V) , C/M , then , the extract was shaken with 0.017 % magnesium chloride to eliminate non – lipid contaminants , according to the method of Folch *et al* (1957) .

This aqueous treatment also cleared off the yellow colors of the C / M extract .In this extract , total lipid (TL) content was determined by the phosphovanillin reaction according to the method of Saifer and

Feldman (1971). Small amount of olive oil was extracted by C / M , then , the extract was evaporated , the residue was then weighed , dissolved in C / M , serially diluted to obtain different concentrations , treated as tissue extract for determination of total lipids , standard curve was plotted (Frings and Dunn , 1970) , from which , TL values were computed. Phospholipid (PL) content of liver tissue was colorimetrically determined in the previous extract , depending on the phosphorus content of PL , without prior acid digestion to avoid inorganic phosphorus – production . Standard curve was prepared from egg yolk by extraction with C / M as aforementioned , then , PL content was colorimetrically estimated using the prepared standard curve (Raheja *et al* , 1973) . For determination of both total cholesterol (TC) and triglyceride (TG) contents , C / M extract was evaporated in boiling water bath , then , the sediment was dissolved in 0.2 % Triton X – 100 (Ide *et al* , 2004) , then , both TC (Roeschlau *et al* , 1974) and TG (Bucolo and David , 1973) were enzymatically determined using commercial colorimetric kits .

Tissue total protein (TP) was extracted with pre-cooled 20 % (W / V) trichloroacetic acid (TCA) , containing 0.2 % Triton X – 100 (as a non-ionic detergent that increases the bulk of the precipitate) , the sediment was dried , shaken with diethyl ether / ethanol (3:1 , V / V) to wash off any lipid impurities , centrifuged , the organic layer was discarded , the sediment was dissolved in one molar sodium hydroxide (Tornqvist and Belfrage , 1976) , then , TP was spectrophotometrically determined using bovine serum albumin as standard (Peters , 1968) .

Statistical analysis :

Data were analyzed by one – way ANOVA , all differences were inspected by Duncan's new multiple – range test . All values were expressed as mean \pm SE (Duncan, 1955). The level of significance was set at less than 0.05

RESULTS

The results shown in Table 1 revealed that liver weight was significantly increased in CCl₄ – treated rats, when compared to control non – treated group. Drug treatment returned liver weight of intoxicated rats near to control , where a non – significant difference

appeared when compared to control ($P > 0.05$). A significant increase in liver tissue content of TL was observed in CCl₄ group .TP significantly declined in this group. Drug treatment returned both values near to normal, although the drug couldn't completely restore protein value to normal level (Figure 1) . In Table 2 , CCl₄ treatment significantly elevated both TC and TG , while it significantly lowered PL . Drug treatment returned these variables to non – significantly different values from control subjects

Table 1 :

Effect of DDB on liver weight and its content of total lipids and total proteins in CCl₄ -treated rats after 30 days of treatment , compared to control . Each value represents $M \pm SE$, (n=10)

Variable	Control	CCl ₄ only	CCl ₄ + DDB
Liver weight (g)	4.30 ± 0.34	5.70 * ± 0.47	4.90 ± 0.31
Total lipids (mg/g wet tissue)	60.2 ± 0.95	79.8 ** ± 1.6	62.3 ± 1.5
Total protein (mg/g wet tissue)	71.3 ± 0.94	50.1 ** $\pm 1/1$	64.2 ** ± 0.86

* $P < 0.05$, ** $P < 0.01$

Table 2:

Effect of DDB on liver content of total cholesterol , triglycerides and phospholipids in CCl₄ -treated rats after 30 days of treatment , compared to control . Each value represents $M \pm SE$, (n=10)

Variable	Control	CCl ₄ only	CCl ₄ + DDB
Total cholesterol (mg / g wet tissue)	5.1 ± 0.37	6.7 ** ± 0.26	5.5 ± 0.14
Triglycerides (mg / g wet tissue)	6.4 ± 0.32	9.6 ** ± 0.15	6.9 ± 0.21
Phospholipids (mg / g wet tissue)	22.2 ± 0.69	19.4 ** ± 0.32	20.8 ± 0.37

* $P < 0.05$, ** $P < 0.01$

DISCUSSIONS

During puberty, lipogenesis is achieved in subcutaneous adipose, liver and perirenal tissues, in addition to other body tissues. However, after puberty, liver becomes the main site for lipogenesis (Gandemier *et al*, 1982). Fatty liver is a consequence of different causative agents, as alcohol, viral hepatitis and many other drugs. Chemical induction of fatty liver was initiated by CCl₄ administration in different experimental models. The changes induced by CCl₄ are quite similar to that of acute viral hepatitis. CCl₄ is metabolized by the cytochrome P-450 system into trichloromethyl free radical that binds to biological membranes initiating lipid peroxidation, disturbed calcium homeostasis and finally cell death (Rechnagel *et al*, 1989). In the present work, CCl₄ administration significantly increased liver tissue weight. Meanwhile, TL content of the liver was significantly elevated with a concomitant inhibition of TP synthesis, manifested as decreased tissue TP content. These observations are in accordance with that of Venukumar and Latha (2002). Inhibition of protein synthesis in the liver was primarily considered to lead to depression of lipoprotein synthesis and accumulation of fat in the liver, leading to fatty liver (Piriou *et al*, 1979). Thus, CCl₄ – induced fatty liver may be – in part – referred to impaired protein synthesis. This hepatotoxin also induced fatty liver through the increase in liver TL content. This effect was also reported by Seakins and Robinson (1963). Treatment with Schisandrin C synthetic analogue (DDB), significantly recovered both liver weight, TL and TP to a non – significant difference from control, although TP didn't return to the control value, but only improved by drug treatment. This probably necessitated a longer treatment period for complete recovery, because DDB treatment was proved in other situations to initiate both protein and glycogen synthesis in CCl₄ – intoxicated rats and protected liver tissue by membrane stabilization (Li, 1991). In the present results, CCl₄ significantly increased both tissue TC and TG, while decreased PL contents. Treatment with DDB, significantly reversed this effect and recovered the three parameters near to normal control values. CCl₄ was known to interfere with the hepatic PL synthesis (Rechnagel *et al*, 1976) and to decrease liver plasma membrane PL content in

both acute and chronic treatments (Camacho and Rubalcava, 1984). The significance of the treatment with DDB in alcoholic fatty liver was proved through its effect on liver TC and TG accumulation, although lower doses than 300 mg / kg body weight couldn't recover CCl₄ nor acetaminophen – induced fatty liver (Kim *et al*, 1999).

Conclusion:

The present observation substantiated the protective potential of a synthetic analogue of Schisandrin C (a natural product from *Schisandra chinensis*) in the treatment of CCl₄ – induced fatty liver. This hepatoprotective value was manifested by the study of additional parameters as TL and PL because previous reports stressed mostly on TG and TC contents in the liver tissue. In addition, study of protein content in conjunction with TL introduced a more satisfactory explanation for the protective action of the drug against both fatty degeneration and hepatomegaly induced by CCl₄ and elucidated that hepatomegaly possibly is induced by an increase in all lipid forms at the expense of protein content; however, DDB treatment could efficiently recover these actions. Thus, DDB can be recommended as an effective medication for fatty liver, which is always – unfortunately – discovered as a sequela for other acute or chronic liver impairments. Adding, the drug also previously introduced a good result in most of liver diseases till hepatocellular carcinoma which is a consequence of viral hepatitis. This makes the drug mostly a more balanced medication for most of liver diseases

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