Comparative study on the protective effect of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin in Hepatitis induced by carbon tetrachloride (CCl_4) in rats

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Abstract: Study on the possible protective effect of (DDB) and Silymarin on Hepatitis induced by CCl_4 was carried out. Injection of CCl_4 daily orally administered to rats in a dose of 2.5ml/kg for three days significantly increase the activity of AST, ALT, ALK. Ph. Bilirubin and GGT by several folds of increase, also urea and creatinin were elevated by CCl_4 given orally. Administration of DDB and Silymarin orally seven day after administration of CCl_4 for three days Significantly decrease liver and kidney enzyme DDB and Silymarin administered before CCl_4 to rats also significantly decrease the activity of liver and kidney enzymes. Histopathological investigation of this study show good confirmation to biochemical analysis. [New York Science Journal 2010;3(9):1-11]. (ISSN: 1554-0200).

Keywords: Hepatitis, DDB, Silymarin, rats.

1. Introduction

Among the several infections that might affect the humn liver are hepatitis viruses A, B, C and D Alter and Mast (1994). Because of its unique metabolism and its intimate relationship to the gastro-intestinal tract, the liver is considered as an important target of toxicity by drugs and xenobiotics.

The degree of hepatotoxicity results from an imbalance between the generation of toxic metabolites and its detoxification processes occurring in the human liver Pineiro-Carrero et al., (2004).

The use of carbon tetrachloride (CCl_4) for induction of liver hepatitis in rat's model was well established (Janakat and Al-Merie 2002; El-Shenawy, 2003).

DDB is synthetic analogue of schizandrin C, one of the active components isolated from Fructus schizandra, a traditional oriental medicinal plant, chemically termed dimethyl 4,4`- dimethoxy- 5,6,5`,6` dimethylene- dioxybiphenyl -2,2` dicaboxylate. This compound (DDB) was shown to protect against liver injury induced by CCl_4 (Oh et al.,2000) . In addition, DDB was used successfully for treatment of cases of chemically induced hepatitis (Kim et al.,2000; El Sawy et al.,2002) , and has a beneficial effect on liver enzymes and the resulting histopathological changes Xu et al.,(1997) .

Silybum marianum (Milk thistle) contains silymarin, a mixture of flavanolignans chiefly consisting of silibin, silydiamin, and silychristine (Wagner,1986). Silybum marianum extracts (usually standardized to contain 70% silymarin) have been shown to protect the liver from wide range of toxins including CCl_4 Vogel et al., (1975).

Silymarin is a well-known plant product, which have hepatoprotective activities that mostly explained by antioxidative properties, inhibition of phosphatidylcholine synthesis or stimulation of hepatic RNA and protein synthesis (Li et al. ; Schumann et al., 2003).

The present study aimed to investigate the protective effect of each of DDB and Silymarin on rats model affected by hepatitis induced by CCl_4.

2. Materials and methods:

1- Materials

1-1 Drugs

Dimethyl Dicarboxylate (DDB) and Silymarin pure materials obtained from Arabic company of medicinal plants (Mebaco, Egypt).

1.2 Chemicals

Carbon teterachloride (CCl4) obtained from Egyptian company for chemicals and pharmaceuticals (ADWIA).

1.3 Diagnostic kits

1- For the determination of transaminases (AST, ALT) obtained from Bio merieux, France.

2- For determination of alkaline phosphates, blood urea nitrogen, creatinine and bilirubin obtained from Biodiagnostic, Egypt.

3-Gamma Glutamic transaminase (GGT) obtained from Quimica Clinica Aplicada S.A, Spain.

1.4 Animals

Forty-eight Sprague dawley albino rats of both six weighting 100g b.wt used through the experiments all animals were obtained from animal house unit national research centre, Dokki Giza, Egypt. The animals allowed free access to water and fed on uniform stander diet formula Rogers (1979).

2- Methods:

2.1- Experimental design

Forty – eight rats were divided into eight groups of six animals each as following:

Group 1- Normal control group received a daily oral dose of 1 ml saline**.**

Group 2- Received a daily oral dose of DDB 300mg/kg for seven days.

Group 3- Received a daily oral dose of Silymarin 22 mg/kg for seven days.

Group 4- Received a daily oral dose of CCl_4 2.5 ml/kg for three days.

Group 5- Received a daily oral dose of CCl_4 2.5 ml/kg for three days followed by given a single oral dose of DDB 300mg/kg for seven days.

Group 6- Received a daily oral dose of CCl_4 2.5ml/kg for three days followed by given a single oral dose of 22mg/kg for seven days.

Group 7- Received a daily oral dose of DDB 300ml/kg for seven days followed by given a single oral dose of CCl_4 25 ml/ kg for three days.

Group 8- Received a daily oral of Silymarin 22 mg/kg for seven days followed by given a single oral dose of CCl4 2.5 mg/kg for three days.

2.2 Assessment of liver and kidney functions :

The blood was obtained from all groups of rats by puncturing rato-orbital plexus Sanford (1954), the blood was allowed to flow into clean dry centrifuge tube and left to stand, and the serum was separated by centrifugation and examined for:

1- AST and ALT were done according to colorimetric method after Reitman and Frnakel (1957).
2- Alkaline phosphates was done calorimetrically after Belfied and Goldberg (1971).
3- Blood urea nitrogen was done according to Henry et al.(1974).
4- Creatinine was done according to colorimetric method (Bartles et al, 1972).
5- Blirubin was done according to Walter and Gerade colorimetric method (1970).
6- Gamma- Glutamic transminase (GGT) was done according to Szasz (1969).

2.3- Histopathological investigation

Tissue specimens form liver and kidney of treated and control rats were fixed in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hematoxyline and eosin.

2.4. Statistical analysis

All results were expressed as mean ± SE comparison between groups were performed by ANOVA followed by Duncan test. $P<0.05$ was considered statistically significant.

According to (Bancroft et al., 1996), the degree of hepatic injury was estimated using an ordinal scale modified from Plaa and Charbonneau (1994).

Table (1): Histological grading of liver injury

Grade	Description
O	No apparent injury by light microscopy
I	Swelling of hepatocytes
II	Ballooning of hepatocyes
III	Lipid droplets in hepatocytes
IV	Necrosis of hepatocytes

3. Results

Results in table (2) shows that CCl_4 significantly increase the activity of AST, ALT, ALK ph., Bilirubin and GGT by several folds of increase. The same effect was observed in case of urea and creatinine.

Table (2): Comparative effect of silymarin (Sy) or biphyenyl dimethyl dicarboxilate (DDB) on liver and kidney toxicity induced by carbon tetrachloride (CCl_4) in rats.

Groups	AST (IU/L)	ALT (IU/L)	ALK PH (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl	GGT (U/L)
Control	$9{\pm}0.34^{a}$	$4.02{\pm}0.063^{a}$	$166.293{\pm}7.43^{b}$	$19.22{\pm}5.3^{a}$	$0.64{\pm}0.033^{a}$	$0.056{\pm}0.016^{a}$	$13.5{\pm}5.7^{a}$
DDB	$7{\pm}0.9^{a}$	$2.2{\pm}0.75^{a}$	$154.59{\pm}4.09^{b}$	$24.47{\pm}2.12^{c}$	$0.56{\pm}0.035^{a}$	$0.23{\pm}0.008^{c}$	$17.6{\pm}13.11^{a}$
Sy	$14.3\ {\pm}3.84^{a}$	$8.5{\pm}4.86^{a}$	$155.366{\pm}10.32^{b}$	$26.92{\pm}1.3^{c}$	$0.6{\pm}0.023^{a}$	$0.03{\pm}0.009^{a}$	$16.4{\pm}5.97^{a}$
CCl_4	$29.2{\pm}2.5^{b}$	$51.2{\pm}5.10^{b}$	$229.267{\pm}5.83^{b}$	$35.04{\pm}3.17^{b}$	$4.46{\pm}0.018^{b}$	$1.55{\pm}0.034^{b}$	$35.36{\pm}7.49^{v}$
CCl_4+DDB	$6.33{\pm}0.88^{a}$	$2.33{\pm}0.67^{a}$	$176.676{\pm}2.16^{c}$	$29.65{\pm}2.16^{c}$	$0.54{\pm}0.04^{a}$	$0.042{\pm}0.016^{a}$	$18.4{\pm}5.8^{a}$
CCl_4+Sy	$8{\pm}0.81^{a}$	$2.6{\pm}0.76^{a}$	$156.466{\pm}6.98^{b}$	$32.47{\pm}4.03^{bc}$	$0.48{\pm}0.05^{c}$	$0.06{\pm}0.029^{a}$	$24.9{\pm}6.44^{C}$
DDB(7) + CCl_4	$18.5{\pm}1.76^{ac}$	$18.5{\pm}6.22^{a}$	$121.875{\pm}6.22^{a}$	$14.65{\pm}0.94^{a}$	$0.65{\pm}0.02^{a}$	$0.36{\pm}0.095^{Cd}$	$28.8{\pm}9.03^{bC}$
Cy (7) +CCl_4	$19.8{\pm}1.24^{ac}$	$39{\pm}3.38^{b}$	$103.384{\pm}5.45^{a}$	$15.31{\pm}2.18^{a}$	$0.58{\pm}0.036^{a}$	$0.43{\pm}0.06^{E}$	

a-c: Means with different letters in the same column differs significantly (P<0.05).

DDB and Silymarin significantly decrease the activity of ALT, AST and ALK ph. before and after administration CCL4.

Meanwhile the decrease was more prominent if the rats pretreated by DDB and Silymarin. The results also in table (2) show that DDB was more effective than Silymarin. From the results shown in table (2) it was a quite obvious that DDB had a significant effect more than Silymarin particularly in case of AST, ALT, urea bilirubin and GGT.

Comparison between the effect of DDB and Silymarin on the activity of liver and kidney enzymes befor injection of CCl_4 was shown table (3).

The same trend was observed in urea and creatinine activities. Figures from 1-7 give more evidence that DDB and Silymarin had a curative and protective effect against liver and kidney damage induced by CCl_4.

Table (3): The differences of the rats affected by DDB and Silymarin before administration of CCl_4.

Parameters	CCl_4	CCl_4 +DDB	CCl_4+ SY
AST (IU/L)	30.5 ± 2.5^{b}	6.82 ± 0.88^{a}	8.7 ± 0.4^{a}
ALT (IU/L)	58.2 ± 5.10^{b}	2.51 ± 0.67^{a}	3.1 ± 0.76^{a}
ALK Ph. (IU/L)	240.5 ± 3.28^{c}	180.33 ± 5.83^{b}	160.4 ± 0.98^{b}
Urea (mg/dl)	37.3 ± 3.17^{b}	30.63 ± 2.16^{c}	29.42 ± 4.03^{bc}
Creatinine (mg/dl)	4.82 ± 0.02^{b}	0.51 ± 0.04^{a}	0.53 ± 0.05^{c}
Bilirubin (mg/dl	1.49 ± 0.03^{b}	0.03 ± 0.02^{a}	0.07 ± 0.03^{a}
GGT (U/L)	36.2 ± 7.49^{b}	17.5 ± 5.80^{a}	22.8 ± 6.44^{c}

a-c: Means with different letters in the same raw differs significantly (P<0.05).

2- Histopatholgical investigation

- **The 1st group (control):**

The animals were apparently normal. Histological examination of liver revealed grade (0) and kidney of this group showed normal structure picture (1,2&3).

- **The 2st group (Received –CCl_4):**

Liver of CCl_4 exposed group showed necrobiotic change of hepatocytes including vacuolar degeneration, nuclear pyknosis and necrosis, the hepatic injury appeared as grade (III, IV). Narrowing of hepatic sinusoids and hyperplasia of Kupper cells were also noticed picture (4). Portal triads showed fibrous connective tissue proliferation and hyperplasia of bile duct picture (5).

Kidney of the same group showed swelling of tubular epithelial ling especially the proximal convoluted tubules. Coagulative necrosis of some renal tubules was also seen picture (6).

- **The 3st group (Received – CCl_4 and Silymarin):**

Liver of animal received- CCl_4 and Silymarin showed ballooning degeneration of hepatocytes and single cell necrosis. Silymarin produced less pronounced hepatoprotective effect and the hepatic injury resembling to grade (II) picture (7). On the other side hyperplasia of bile duct in for m of numerous number of newly formed bile ductless picture (8).

Kidney of the same group revealed mid swelling of tubular epithelial lining in compression with the 2nd group picture (9).

- **The 4st group (Received- CCl_4 and DDB):**

Liver of animals received – CCl_4 and DDB showed mild swelling of hepatocytes and narrowing of hepatic sinusoids. DDB induced more hepatoprotection than Silymarin and the tissue injury appeared as grade (1) picture (10). Portal triads showed normal histological structure as well as kidney in compression with the 1st group (control) pictures (11, 12).

4. Discussion

This study shows that, in rats, treatment with Biphyenyl Dimethyl Dicarboxylate (DDB) and Silymarin inhibited CCl_4 induced hepatic and kidney damage. Liver damage was evaluated by measurement of ALT, AST, ALK ph., Bilirubin and GGT activities and kidney damage was evaluated by measurement of urea and creatinine activities. Moreover treatment of CCl_4 injected rats with DDB and Silymarin before and after the administration of CCl_4 improve the activities of liver and kidney enzymes.

In the present study, intoxication with CCl_4 caused drastic increase in the activities of liver and kidney enzymes. But the rats orally administered with DDB and Silymarin for three days after administration of CCl_4 was more effective if administered before CCl_4 injection.

Vogal et al. (1975) showed that Silymarin in the most potent protecting substance it cause marked reduction in the activities of several liver enzyme sin experimental animals. Li et al.(2003) stated that Silymarin is able to reduce ALT elevation in animals exposed to CCl_4. Schumann et al.,(2003) stated that silibinine is the major pharmacologically active compound of Silymarin marianum fruit extracts Silymarin its well known hepatoprotecitve activities are mostly explained by antioxidative properties, inhibition of phosphatidycholine synthesis or stimulation of hepatic RNA and protein synthesis. This exemplifies the heapatoprotective potential of Silibinine as an immune modifier in T-cell dependent hepatitis in vivo.

Concerning the protective effect of DDB (Xu et al., 1997) reported that DDB efficiently protected the hepatocytes against CCl_4 induced damage. Wagner (1986) stated the DDB- dependently decreased the levels of ALT and AST compared with CCl_4 intoxication only.

Also he stated that DDB cause significant decrease in the elevated liver enzymes in chemically injured rats.

The results of histopathological investigation of the present study show good confirmation of the biochemical analysis.

Conclusion

The results in the presented study indicate that DDB and Silymarin improve the activates of liver and kidney enzymes of both normal and CCl_4 intoxicated rats meanwhile it was observed that DDB was more effective than Silymarin.

Moreover this study showed that the curative effects of these compounds are a little more effective than its protective effect against CCl_4 induced liver toxicity.

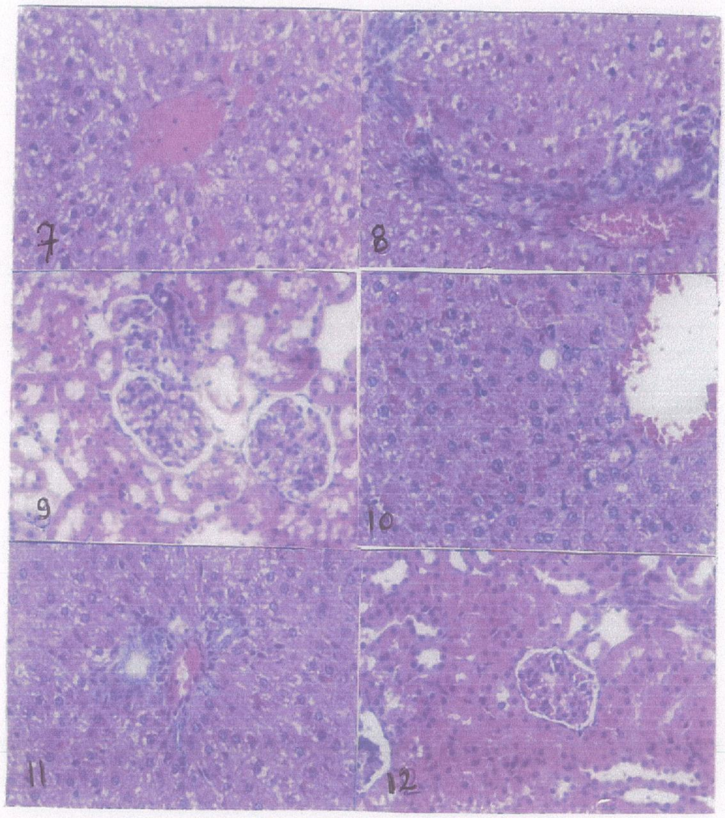

Fig. (7)- Liver of CCL_4 & Silymarin treated group showing ballooning degeneration of hepatocytes (H&E X200).

Fig. (8)-Liver of CCL_4 & Silymarin treated group showing marked hyperplasia of bile duct (H&E X200).

Fig. (9)-Kidney CCL_4 & Silymarin treated group showing mild swelling of tubular epithelial lining (H&E X200).

Fig. (10)-Liver of CCL_4 & DDB treated group showing mild swelling of hepatocytes (H&E X200).

Fig. (11)-Liver of CCL_4 & DDB treated group showing normal histological structure of portal triad (H&E X200).

Fig. (12)-Kidney of CCL_4 & DDB treated group showing normal histological structure (H&E X200).

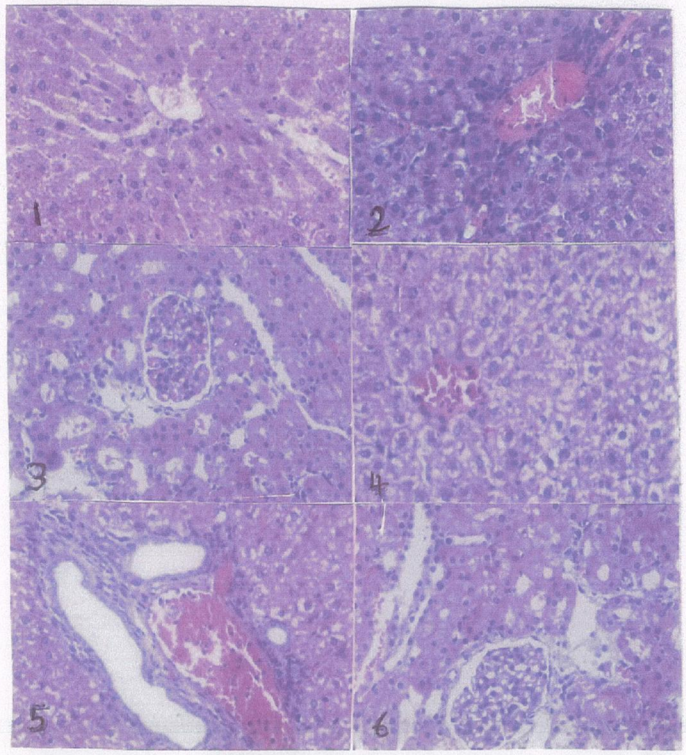

Fig. (1)- Liver of control group showing normal histological structure of it is hepatic lobule (H&E X200).
Fig. (2)- Liver of control group showing normal histological structure of it is portal triad (H&E X200).
Fig. (3)- Kidney of control group showing normal histological structure of it is parenchyma (H&E X200).
Fig. (4)- Liver of CCL_4 – exposed group showing necrobiotic changes of it is hepatocytes (H&E X200).
Fig. (5)- Liver of CCL_4 – exposed group showing proliferation of fibrous connective tissue and hyperplasia of bile duct (H&E X200).
Fig. (6)- Kidney CCL_4 – exposed group showing swelling of tubular epithelial lining (H&E X200).

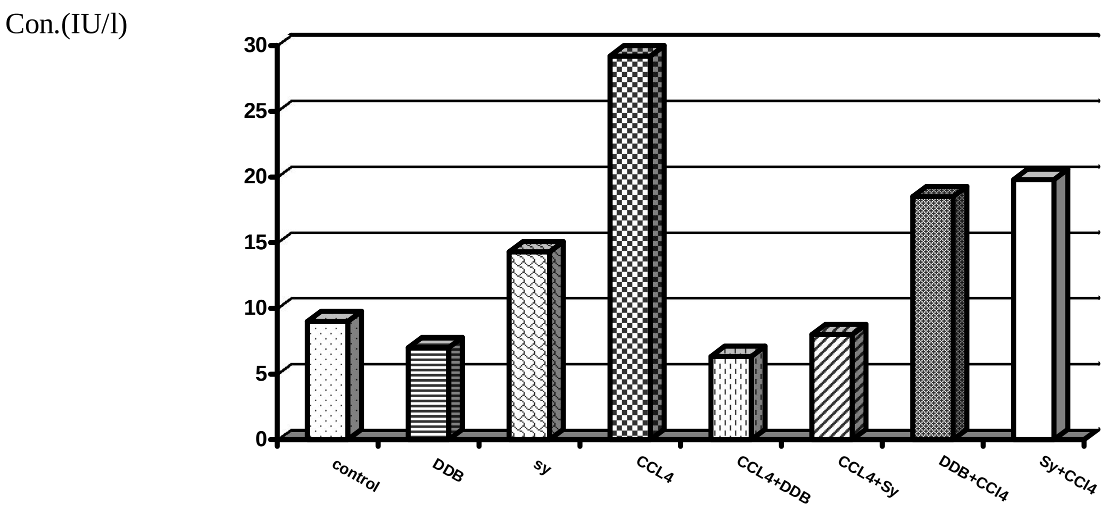

Fig (1): Effect of Sy and DDB on the activity of AST on heptatotoxicity induced by CCl_4 in rats (n -6).

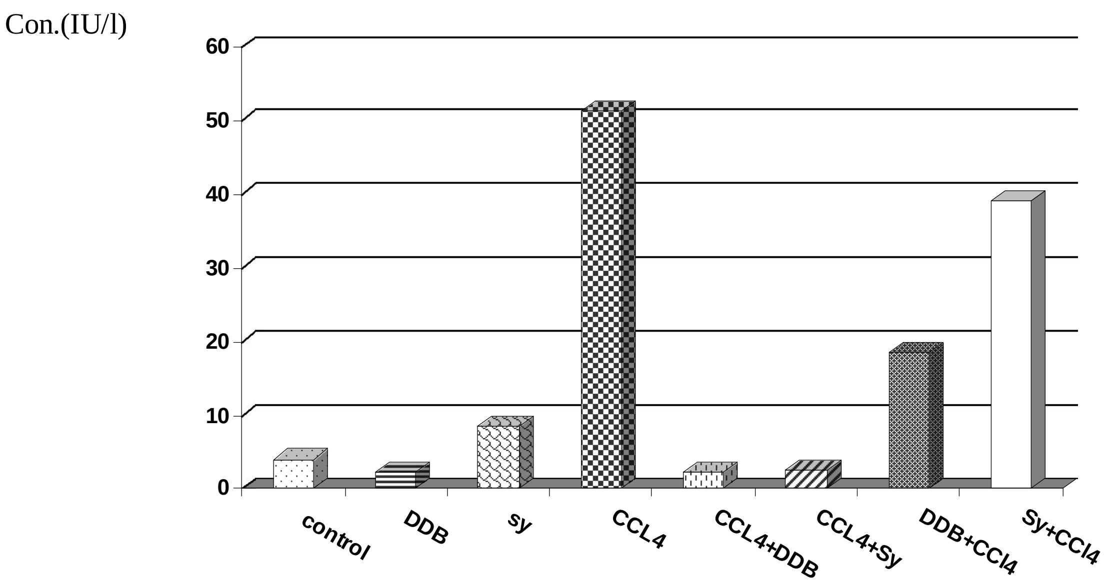

Fig (2): Effect of Sy and DDB on the activity of ALT on heptatotoxicity induced by CCl_4 in rats (n -6).

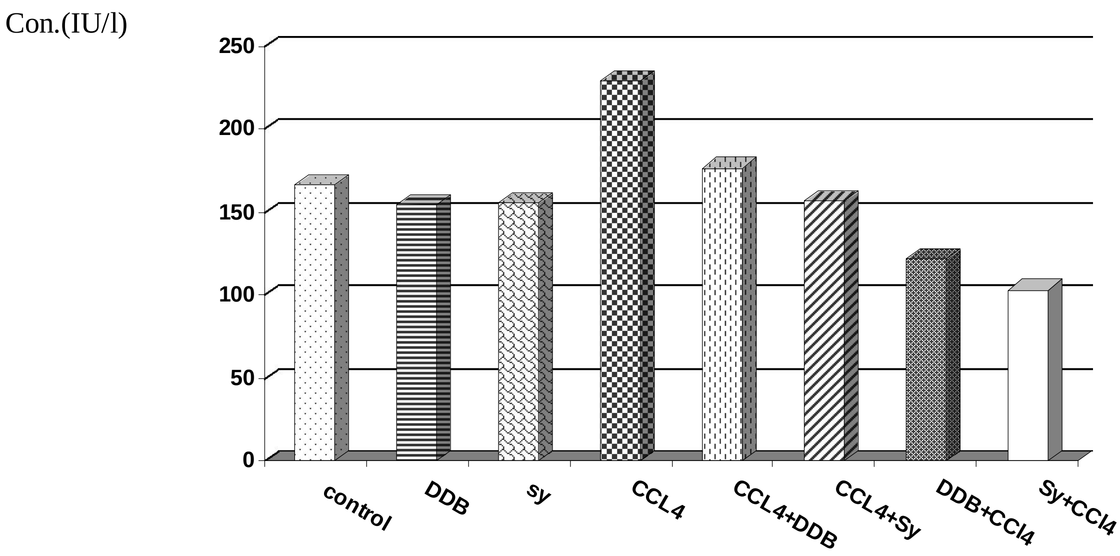

Fig (3): Effect of Sy and DDB on the activity of Alkaline phosphatase on heptatotoxicity induced by CCl_4 in rats (n -6).

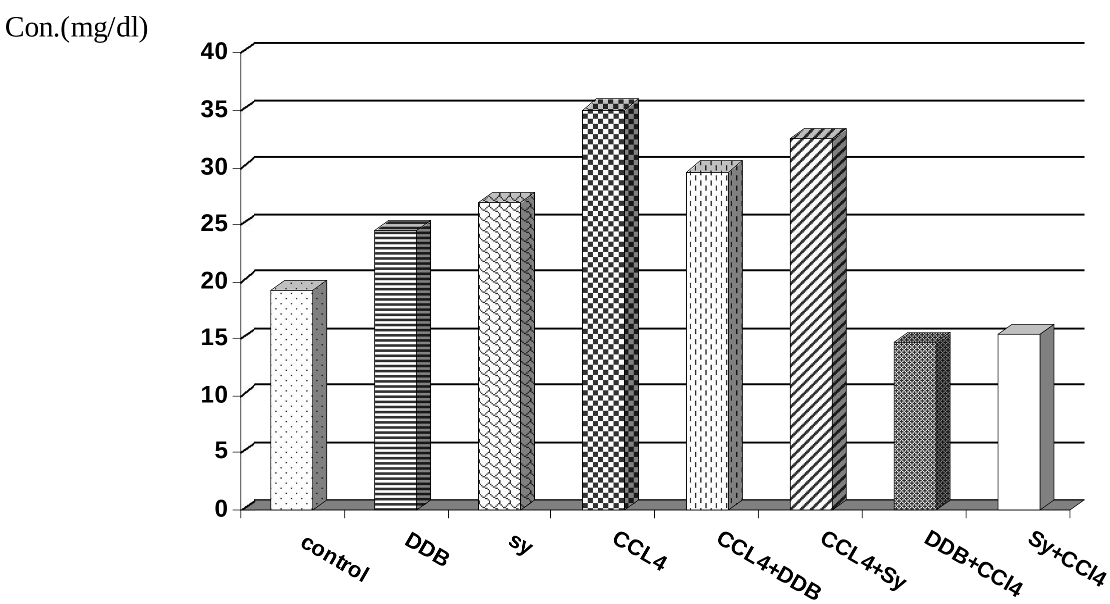

Fig (4): Effect of Sy and DDB on the activity of urea on heptatotoxicity induced by CCl_4 in rats (n -6).

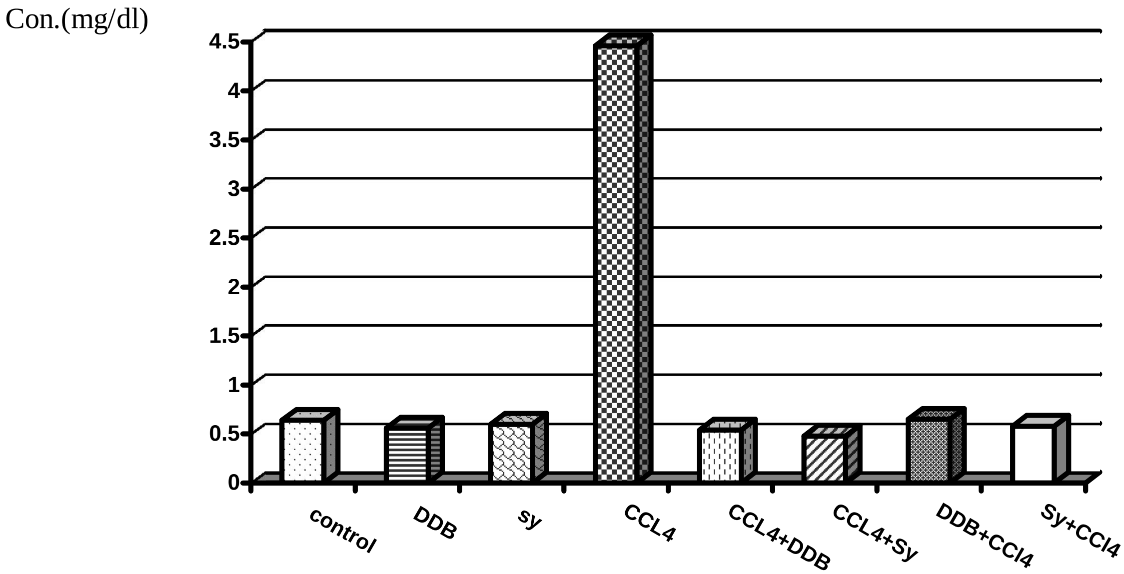

Fig (5): Effect of Sy and DDB on the activity of creatinine on heptatotoxicity induced by CCl_4 in rats (n -6).

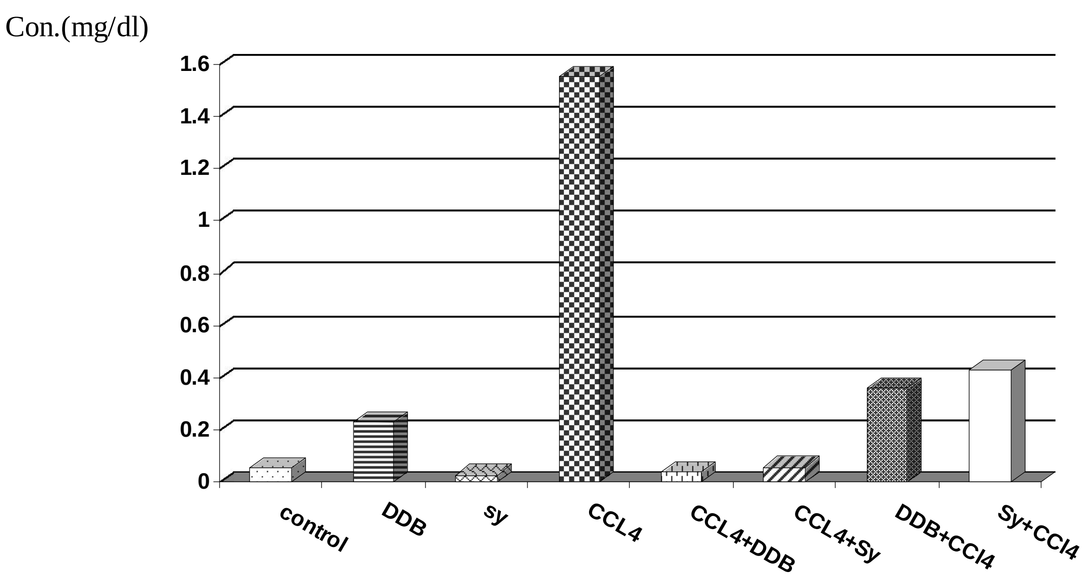

Fig (6): Effect of Sy and DDB on the activity of bilirubin on heptatotoxicity induced by CCl_4 in rats (n -6).

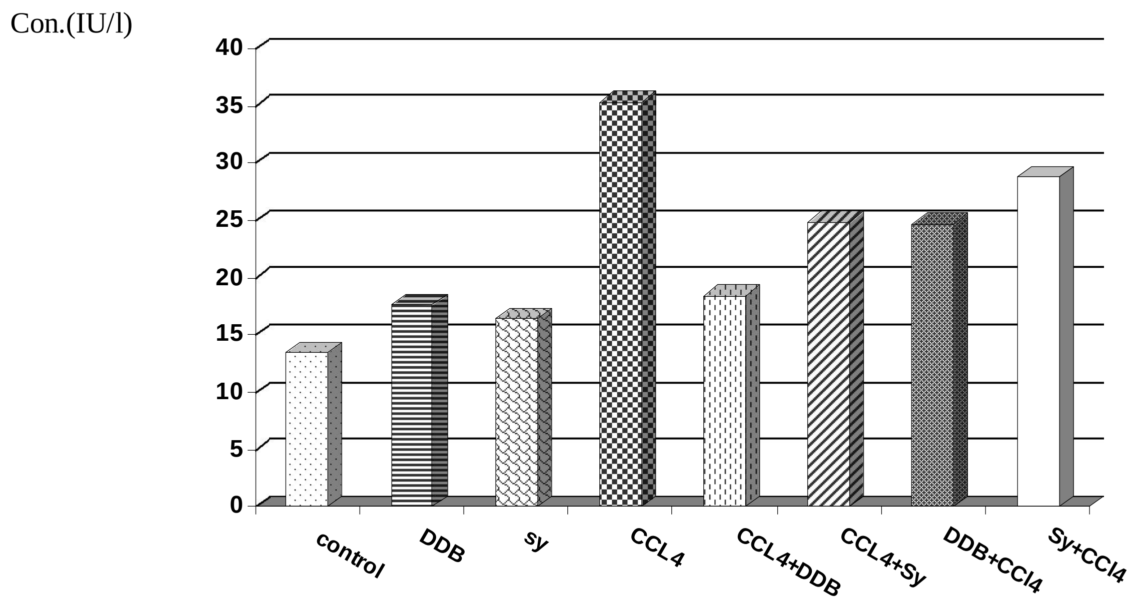

Fig (7): Effect of Sy and DDB on the activity of GGT on heptatotoxicity induced by CCl_4 in rats (n -6).

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