



# Protective role of dimethyl diphenyl bicarboxylate (DDB) against erythromycin induced hepatotoxicity in male rats

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## Abstract

In this study, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate (DDB) was examined to justify its role in the hepatoprotection against erythromycin toxicity in male rats. Oral daily administration of toxic dose of erythromycin stearate (EE, 100 mg/kg body weight) was given to male rats for fourteen days to induce hepatotoxicity. It was found at the end of the experiment (14 days) that the total body weight was markedly decreased in rat treated with erythromycin stearate (EE). Hepatomegaly and splenomegaly were recorded in rats treated with erythromycin stearate (EE). The red blood cells (RBCs) count, haemoglobin content (Hb) and haematocrit value (Hct) were significantly reduced in rats treated with EE. The hepatotoxicities were monitored by increased level of plasma enzymes (aspartate aminotransferase; AST and alanine aminotransferase; ALT), total bilirubin, direct bilirubin, cholesterol, total lipids and glucose. The data obtained showed that oral administration of DDB (100 mg/kg body weight) has significantly prevented the occurrence of EE-induced liver damage. The biochemical data were supplemented by histopathological examination of the liver of control and treated rats. DDB showed a better hepatoprotective effect compared with ursodesoxycholic acid or Silymarin (Sil), as a reference drug.

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## 1. Introduction

Schisandrin B (Sch B) is an active dibenzocyclooctadiene derivative isolated from the fruit of a Chinese herb (*Schisandra chinensis*) commonly used for the treatment of hepatitis (Hancke et al., 1999). Extensive studies have shown that Sch B pretreatment protects against carbon tetrachloride-induced hepatotoxicity, myocardial ischemia/reperfusion injury and brain oxidative damage in rodents (Ip et al., 1995; Ko and Lam, 2002; Chiu et al., 2003). A structure–activity relationship study indicated that dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate, DDB (Fig. 1), which lack the cyclooctadiene ring, did not stimulate mitochondrial glutathione

status nor did protect carbon tetrachloride-induced hepatotoxicity in mice (Ip et al., 2000).

DDB has been used as a curative agent for patients with acute or chronic viral hepatitis (Lee et al., 1991; Kim et al., 2000; Park et al., 2005). It is a synthetic derivative of schisandrin C, which is present as a component of *Fructus schizandrae*. Hepatoprotective effects of DDB were reported against a variety of toxicants (Lee et al., 1991; Liu et al., 1994; Kim et al., 1995). Nowadays, DDB is used as a hepatoprotective agent for human patient with viral hepatitis in several countries. Also, it is used in case of veterinary medicine (Helal et al., 2003). On the other hand, Sil (reference hepatoprotective drug) a group of milk thistle (*Silybum marianum*) flavonoids is used as a producer of liver protector (El-Samaligy et al., 2006).

A decrease in cellular reduced glutathione (GSH) level enhances oxidative stress as GSH consumes the oxygen free radical (Park et al., 2005). Deficiency of cellular GSH

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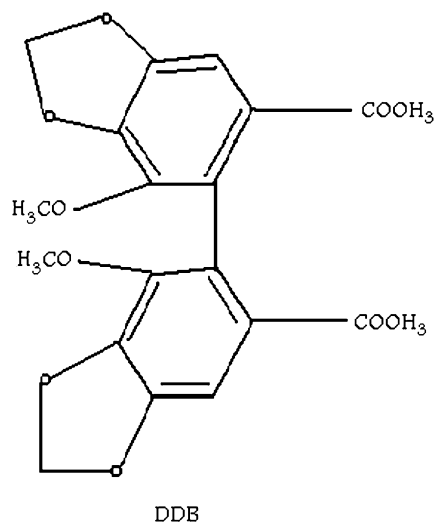


Fig. 1. Chemical structure of DDB.

increases prooxidant production, and promotes apoptosis (Kim et al., 2003; Park et al., 2005). Disruption of GSH system by decreasing its content stimulates cellular proteins, which could enhance susceptibility of cells to toxic insults (Park et al., 2005). The hepatoprotection of DDB and schisandrins may attribute to the stimulation of hepatic mitochondrial GSH antioxidant system by activation of GSH related enzyme; as indicated by increased tissue GSH level (Ip et al., 1995).

The antibiotics are extensively used against wide spectrum microorganisms. Erythromycin is a commonly used antibiotic, where at high doses, it induces severe liver injury and cardiovascular dysfunction both in humans and experimental animals (Richard and Adams, 1976; Pari and Murugan, 2004).

Sivaraman et al. (2005) provided a model of acute and chronic liver toxicity induced from exposure to drugs or environmental agents; and as a disease model for the study of viral hepatitis infection and cancer metastasis. Hepatotoxicity induced by erythromycin was detected by increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, and cholesterol in the blood plasma of female white rats (Helal et al., 2003; Pari and Murugan, 2004). Pessayre et al. (1985) reported that over doses of erythromycin will led to occurrence of hepatitis, hepatic dysfunction, jaundice and necrosis of hepatocytes.

The present study was undertaken to assess the hepatotoxicity of erythromycin and to demonstrate the protective effect of DDB on male white rats. The obtained data were compared with those of Sil as a standard hepatoprotective drug.

## 2. Materials and methods

### 2.1. Drugs

Erythromycin stearate (EE) and Sil were purchased from local pharmacy, as they are prepared by Khaira phar-

maceutical and chemistry Industry Company, Cairo, Egypt. DDB was also purchased from the local pharmacy, but was imported from Beijing union pharmaceutical factory, Beijing, PR China.

### 2.2. Animals

Apparently healthy male white rats (*Epyomis norvegicus*) weighing 215–260 g were provided from AbouRawach zone, Giza Governorate, and laboratory acclimatized for one week. The research was performed in compliance with internationally accepted guidelines on animal use in research (NIH guide, 1996). The animals were fed on standard pellet diet (purchased from local supplier) and given water ad libitum.

### 2.3. Experimental protocol

The experiment was started on the first of February, 2006 and ended by the 14th day of the same month. The animals were randomly segregated into five groups, consisting of seven animals each. The experimental groups were proceeding as follows:

- Group 1: Control male rats.
- Group 2: Rats orally treated with EE (100 mg/kg body weight) in aqueous solution for 14 days. EE dose was conducted based on Helal et al. (2003).
- Group 3: Rats orally treated with DDB (100 mg/kg body weight) in aqueous solution for 14 days. DDB dose was conducted, based on Park et al. (2005).
- Group 4: Rats orally treated with EE (100 mg/kg body weight) and DDB (100 mg/Kg body weight) in aqueous solution for 14 days.
- Group 5: Rats orally treated with EE and reference drug Sil (200 mg/kg body weight) in aqueous solution for 14 days. Sil dose was conducted based on Pari and Murugan (2004).

The animals were weighed at the first and at the end of the experiment. Also, the weights of the liver and spleen of each rat were recorded and their percentage to the total body weight was calculated as indices.

### 2.4. Blood sampling

At the end of 14 days, the animals were sacrificed by cervical breaking. Blood was collected from the heart in a lithium heparinized tube to prevent blood clotting.

### 2.5. Blood parameters

Red blood cells (RBCs) and total white blood cells (WBCs) were counted by haemocytometer Neubauer slide, using 0.9% NaCl and Gentiana violet as diluting fluids. Haemoglobin content (Hb, g/100 ml) was determined following the method recommended by Henry (1964). The

precision and accuracy measure  $\pm 0.1$  g/100 ml. The calculated blood indices (MCH, MCHC and MCV) were computed as per the following formulae:

$$\text{MCH (pg/cell)} = \text{Hb (g/100 ml)} / \text{RBCs in millions} \times 10.$$

$$\text{MCHC (g/100 ml)} = \text{Hb (g/100 ml)} / \text{Hct (\%)} \times 10.$$

$$\text{MCV (\mu m}^3\text{)} = \text{Hct (\%)} / \text{RBCs in millions} \times 10.$$

## 2.6. Biochemical analysis

The blood plasma was separated after centrifuging the blood sample at 5000 rpm for 5 min and the following biochemical metabolites of blood plasma and enzymes were quantified: the activities of aspartate aminotransferase; AST and alanine aminotransferase; ALT (accuracy level  $\pm 0.1$  U/l) (Reitman and Frankel, 1957), total bilirubin (accuracy level  $\pm 0.1$  mg/100 ml) (Malloy and Evelyn, 1937), total lipids (accuracy level  $\pm 0.2$  g/100 ml) (Schmit, 1964), blood glucose (accuracy level  $\pm 2.0$  mg/100 ml) (Trinder, 1969), cholesterol (accuracy  $\pm 3.0$  mg/100 ml) (Stein, 1986), total proteins (accuracy level  $\pm 0.2$  g/100 ml) (Henry, 1964) and albumin (accuracy level  $\pm 0.07$ – $0.12$  g/100 ml) (Dumas and Biggs, 1972). Globulins level was computed as a difference between the total proteins and albumin content.

## 2.7. Histopathology

Liver tissue slices were fixed in 10% formalin. The hepatocytes profiles were assessed by making paraffin section slides of the liver. Four  $\mu\text{m}$  paraffin sections were stained with haematoxylin and eosin (H&E). Histopathological examinations of the samples were done in blinded slides. Five fields were viewed for histopathological signs at a magnification of  $400\times$ . Events of central necrosis, hepatocyte degeneration and portal inflammation were graded following the idea of Park et al. (2005) as the following 0: negative findings; (1) evidence of pathological changes; (2) mild pathological changes; (3) moderate pathological changes; and (4) marked pathological changes.

## 2.8. Statistical analysis

The obtained raw data in each experimental group were computed into mean and standard deviation. Significance test (*t*-test) was computed between the control and treated groups (Pipkin, 1984).

## 3. Results

### 3.1. Total body weight, liver and spleen indices

The changes of the total body weight of the control rats showed a non-significant reduction. It was reported that rats treated with EE had a significantly reduced body weight, compared with the initial body weight (Table 1). DDB treatment did not significantly affect the total body weight of the rats. Combined treatments of EE along with DDB or EE along with Sil induced non-significant differences of the total body weight. The data recorded general increased liver index of treated rats compared with the control one. Rats treated with EE, EE along with DDB and EE along with Sil showed significant rise of the liver index over the control rats, whereas rats treated with DDB alone exhibited a non-significant rise of the liver index (Table 1). The spleen index showed a significant rise of rat treated with EE. Combined treatment of EE with DDB or Sil induced non-significant effect on spleen index.

### 3.2. Blood parameters

The data recorded in Table 2 showed that EE treatment induced a significant decrease of the RBCs count, Hb content and Hct values of male rats compared to the control. Treatment with DDB alone or in combination with EE induced non-significant differences of the tested blood parameters. Similar results were recorded for rats treated with Sil along with EE. The calculated blood indices, MCH and MCHC, were non-significantly decreased in all treated groups, compared with the control group value, whereas a significant reduction was recorded for EE-treated rats. The erythrocytes MCV of EE-treated rats were non-significantly reduced, whereas those of rats treated with DDB or Sil showed values close to the value of the control one. The total WBCs count was non-significantly amplified in all the experimental groups.

### 3.3. Biochemical parameters

EE treatment induced significant increase in the activities of plasma AST and ALT (Table 3). Also, the total bilirubin, glucose, cholesterol, total proteins, and globulins were significantly elevated after EE treatment, whereas the total lipids and albumin in the blood plasma were significantly reduced as a result of EE treatment.

Table 1  
Changes in the body weight (g) and liver and spleen indices (%) of un-treated and treated rats

Item		Control	EE	DDB	EE + DDB	EE + Sil	No.
Body weight	Zero time	240.101 $\pm$ 10.0	260.101 $\pm$ 12.10	235.211 $\pm$ 9.243	215.10 $\pm$ 10.12	225.12 $\pm$ 12.123	7
	14 days	235.17 $\pm$ 11.120	205.13* $\pm$ 7.124	237.421 $\pm$ 10.124	210.11 $\pm$ 10.421	229.14 $\pm$ 11.121	7
Liver index	14 days	2.667 $\pm$ 0.121	2.927* $\pm$ 0.023	2.785 $\pm$ 0.124	3.012* $\pm$ 0.214	2.813* $\pm$ 124	7
Spleen index	14 days	0.500 $\pm$ 0.010	0.585* $\pm$ 0.024	0.512 $\pm$ 0.041	0.476 $\pm$ 0.23	0.513 $\pm$ 0.034	7

\* Significant at  $P \leq 0.05$ .

Table 2  
Changes in the blood parameters of control and treated rats for 14 days

	Control	EE	DDB	EE + DDB	EE + Sil	No.
RBCs ( $\times 10^6/\text{mm}^3$ )	6.402 $\pm$ 0.423	4.352* $\pm$ 0.834	6.567 $\pm$ 0.567	6.387 $\pm$ 0.698	6.213 $\pm$ 0.567	7
Hb (g/100 ml)	9.891 $\pm$ 0.793	6.263* $\pm$ 0.673	9.672 $\pm$ 0.563	9.273 $\pm$ 0.923	9.103 $\pm$ 0.692	7
Hct (%)	41.232 $\pm$ 3.124	29.123* $\pm$ 5.921	44.021 $\pm$ 4.927	40.013 $\pm$ 4.321	39.029 $\pm$ 6.012	7
MCH (pg/cell)	15.45 $\pm$ 1.51	14.39 $\pm$ 1.79	14.74 $\pm$ 1.9	14.53 $\pm$ 1.72	14.65 $\pm$ 1.66	7
MCHC (g/100 ml)	2.4 $\pm$ 0.41	2.15 $\pm$ 0.31	2.19 $\pm$ 0.23	2.32 $\pm$ 0.14	2.33 $\pm$ 0.16	7
MCV ( $\mu\text{m}^3$ )	64.42 $\pm$ 6.12	66.94 $\pm$ 7.12	67.03 $\pm$ 8.14	62.64 $\pm$ 7.61	62.8 $\pm$ 6.92	7
WBCs ( $\times 10^3/\text{mm}^3$ )	6.40 $\pm$ 0.72	7.84 $\pm$ 0.561	6.612 $\pm$ 0.621	6.921 $\pm$ 0.812	6.801 $\pm$ 0.621	7

No.: number of animals.

EE: erythromycin stearate (100 mg/kg).

DDB: dimethyl diphenyl bicarboxylate (100 mg/kg).

EE + DDB: 100 mg/kg EE + 100 mg/kg DDB.

EE + Sil: 100 mg/kg EE + 200 mg/kg Silymarin.

\* Significant at  $P \leq 0.05$ .

Table 3  
Changes in some biochemical parameters of control and treated rats for 14 days

	Control	EE	DDB	EE and DDB	EE and Sil	No.
AST (U/L)	27.713 $\pm$ 1.47	51.43* $\pm$ 1.624	27.121 $\pm$ 0.963	29.142 $\pm$ 2.419	30.116 $\pm$ 0.467	7
ALT (U/L)	21.517 $\pm$ 0.873	41.512* $\pm$ 1.591	22.046 $\pm$ 1.462	23.501 $\pm$ 9.972	23.774 $\pm$ 2.951	7
Total bilirubin (mg/100 ml)	0.886 $\pm$ 0.192	2.163* $\pm$ 0.371	0.952 $\pm$ 0.131	1.091 $\pm$ 0.212	1.0427 $\pm$ 0.664	7
Direct bilirubin (mg/100 ml)	0.173 $\pm$ 0.012	0.189 $\pm$ 0.032	0.181 $\pm$ 0.023	0.174 $\pm$ 0.016	0.172 $\pm$ 0.021	7
Total lipids (g/100 ml)	11.562 $\pm$ 1.201	6.692* $\pm$ 1.692	10.621 $\pm$ 1.591	12.011 $\pm$ 1.969	7.596* $\pm$ 1.569	7
Glucose (mg/100 ml)	110.121 $\pm$ 2.101	250.412* $\pm$ 10.121	115.521 $\pm$ 9.627	150.121* $\pm$ 2.120	170.141* $\pm$ 15.129	7
Cholesterol (mg/100 ml)	159.165 $\pm$ 12.125	205.412* $\pm$ 14.124	157.512 $\pm$ 12.611	140.112 $\pm$ 13.121	168.124 $\pm$ 12.121	7
Total proteins (g/100 ml)	9.355 $\pm$ 0.104	9.886* $\pm$ 0.241	9.261 $\pm$ 0.256	9.421 $\pm$ 0.568	9.021 $\pm$ 0.467	7
Albumin (g/100 ml)	4.968 $\pm$ 0.316	4.328* $\pm$ 0.217	4.689 $\pm$ 0.542	4.478* $\pm$ 0.124	4.121* $\pm$ 0.512	7
Globulins (g/100 ml)	4.387 $\pm$ 0.401	5.558* $\pm$ 0.201	4.572 $\pm$ 0.327	4.943* $\pm$ 0.162	4.921* $\pm$ 0.152	7

No.: number of animals.

EE: erythromycin stearate (100 mg/kg).

DDB: dimethyl diphenyl bicarboxylate (100 mg/kg).

EE + DDB: 100 mg/kg EE + 100 mg/kg DDB.

EE + Sil: 100 mg/kg EE + 200 mg/kg Silymarin.

\* Significant at  $P \leq 0.05$ .

The mixed treatment of EE and DDB induced non-significant changes of the levels of AST, ALT, total bilirubin, total lipids and cholesterol. On the other hand, the glucose, albumin, and globulins content were significantly increased after the EE along with DDB treatment.

It was found that the levels of AST, ALT, total bilirubin, direct bilirubin, cholesterol and total proteins did not significantly differ between rats treated with the mixture of Sil and EE and control rats. In the same treatment, value of the total lipids, glucose and globulins content were significantly increased, whereas, the albumin content was significantly decreased in treated rats compared with the control (Table 3). It was found that, DDB treatment did not induce any significant differences of the tested biochemical parameters (Table 3).

### 3.4. Histopathology

Healthy control rats showed no histopathological changes in the liver (Fig. 1 and Table 4), whereas, treatment with EE induced hepatic necrosis, hepatocellular

Table 4  
Histopathological activity index in the liver of control and treated rats for 14 days

Treatment	Central necrosis	Hepatocyte degeneration	Inflammation	No.
Control	0	0	0	7
EE	3.4 $\pm$ 0.5*	3.4 $\pm$ 0.4*	2.7 $\pm$ 0.7*	7
DDB	0	0	0	7
EE + DDB	0	0.9 $\pm$ 0.01	0	7
EE + Sil	1.0 $\pm$ 0.5*	1.4 $\pm$ 0.5*	1.0 $\pm$ 0.6*	7

No.: number of animals.

EE: erythromycin stearate (100 mg/kg).

DDB: dimethyl diphenyl bicarboxylate (100 mg/kg).

EE + DDB: 100 mg/kg EE + 100 mg/kg DDB.

EE + Sil: 100 mg/kg EE + 200 mg/kg Silymarin.

Hepatic injuries were graded as 0: negative findings; 1: evidence of pathological changes; 2: mild pathological changes; 3: moderate pathological changes; and 4: marked pathological changes.

\* Significant at  $P \leq 0.05$ .

damage and infiltration of inflammatory cells (Fig. 2 and Table 4). The liver histopathology of rat treated with DDB along with EE or with Sil along with EE showed

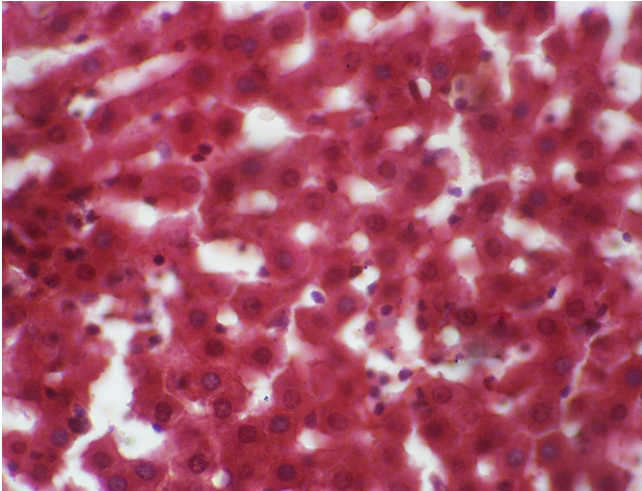


Fig. 2. Photograph of normal rat liver (H&E 400×).

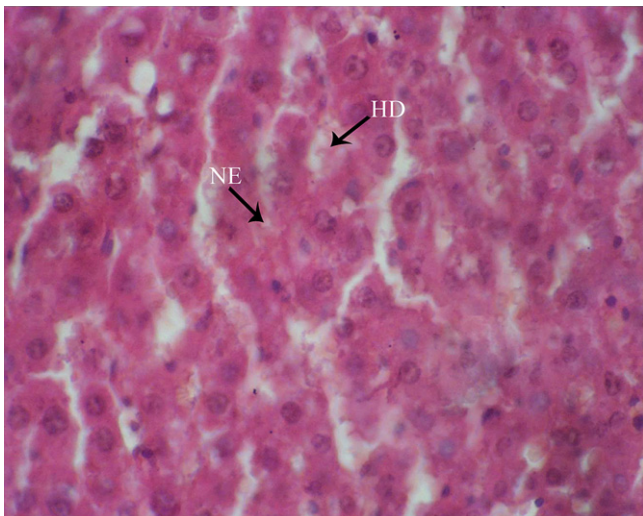


Fig. 3. Photograph of rat liver treated with erythromycin stearate (H&E 400×) showing necrosis (NE) and hepatocyte degeneration (HD).

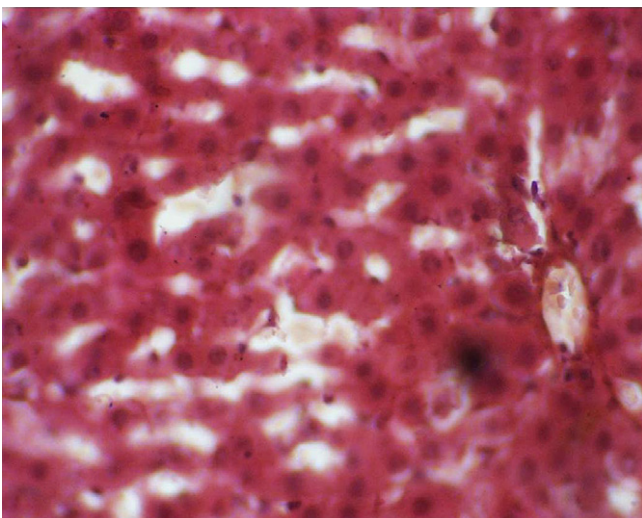


Fig. 4. Photograph of rat liver treated with DDB (H&E 400×) showing normal structure.

few degenerated cells (Fig. 4 and Table 4). The liver histopathology of rat treated with DDB alone showed a normal profile (Fig. 3 and Table 4).

By examining the histological activity index (HAI) of the liver, it was found that rats treated with EE showed moderate pathological changes (necrosis and cells degeneration) and mild inflammation, zero histopathological signs were recorded for both control and DDB treated rats. The combined treatment of EE and DDB showed few degenerated cells, whereas, Sil and EE mixed treatment showed histopathological score 1.0, 1.4 and 1.0 for central necrosis, hepatocyte degeneration and inflammation, respectively; which is significantly different from the control group. Thus, the mutual treatment of DDB and EE significantly reduces the histopathological effects (Table 4) (see Figs. 5 and 6).

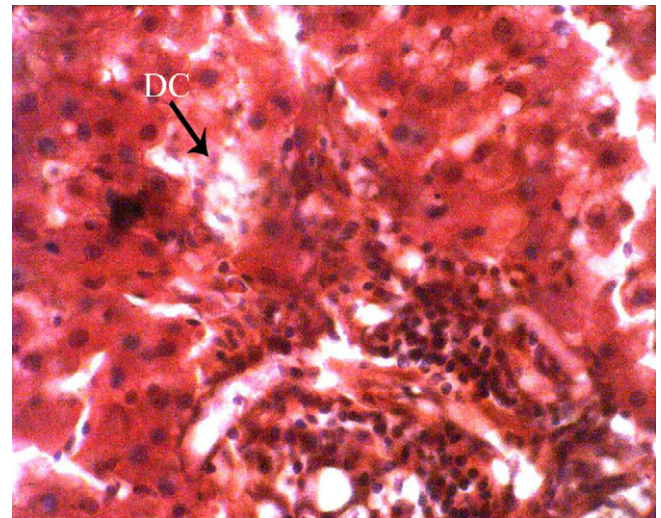


Fig. 5. Photograph of rat liver treated with erythromycin stearate and DDB (H&E 400×) showing few degenerated cells (DC).

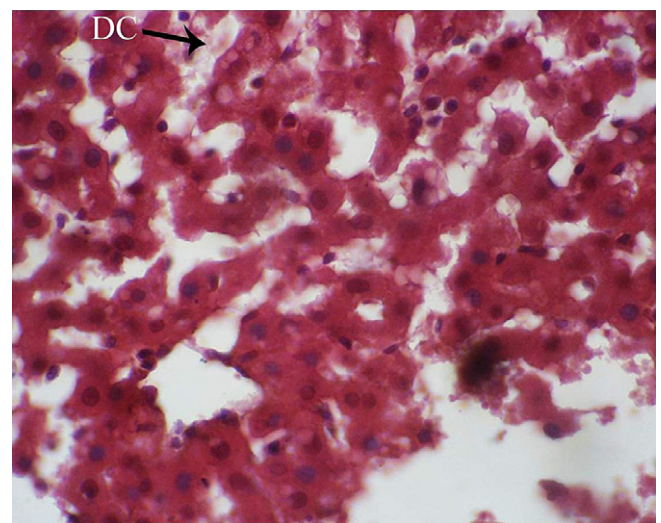


Fig. 6. Photograph of rat liver treated with erythromycin stearate and silymarin (H&E 400×) showing mild degenerative effect (degenerated cells DC).

#### 4. Discussion

Nowadays, antibiotics are extensively used for the treatment of many microbial infections or after the surgical operations. In Egypt, erythromycin is used in veterinary practice as well as in human medication. Erythromycin stearate (EE) was toxic at 0.1 mmol/kg, inducing cellular degeneration accompanied by increased number of secondary lysosomes (Cox et al., 1988).

The hepatotoxicity and hepatitis induced by many drugs or viral infection are predominantly reported (Biagini et al., 2006). The present study dealt with the use of DDB or Sil drugs for treatments of EE induced hepatotoxicity in male rats.

In the present experiment, EE treatment of male rats induced significant fall in the body weight after 14 days, compared to the same group before treatment. This means that EE-treated rats suffered from loss of appetite, as similarly reported by Helal et al. (2003). In contrast, DDB treatments alone or in combination with EE induced non-significant change in the total body weight. Also, rats treated with the combination of Sil and EE were non-significantly increased after 14 days of treatment. Treatment with DDB or Sil improved the body weight loss caused by EE treatment. This may be due to the fact that these drugs exerted antioxidant and hepatoprotective effects (Helal et al., 2003 and Park et al., 2005). The EE treatment induced significant increase of the liver and spleen indices, compared with those of control rats. This may be attributed to the degenerative effects induced in these organs and loss of body weight. Treatment with DDB or Sil along with EE did not restore the liver weight to the range of control rats, which is an indication of severe hepatic effects. Meanwhile, the spleen of rat treated with DDB or Sil along with EE was non-significantly changed from the control value. This could be possibly related to the fact that the liver is a target organ of metabolism that is highly supplied with blood, while the spleen is not so.

A significant reduction of RBCs, Hb and Hct values of rats treated with EE observed in this study may reflect the adverse effects induced in the haemopoietic organs. The decreased RBCs count value may be related to inhibition of erythropoiesis or decreased flow rate of RBCs from the spleen. The reduction of Hct value and Hb content observed in EE-treated rats may reflect the anemic state as a result of drug action. Helal et al. (2003) recorded reduced Hb content in rats treated with erythromycin. The induced anemic state after EE treatment could be attributed to the release of immature RBCs, so that the Hct value was also reduced. The reduction of MCH and MCHC in rats treated with EE is an indication of anemia induced by EE, while the rise of MCV after EE treatment could possibly reflect the swelling of RBCs which is finally broken down. This result coincides with the reduction of RBCs recorded in the present study, whereas treatment with DDB or Sil along with EE restored the blood parameters to the control level. Also, DDB treatment did not induce any significant change of blood parameters.

The total WBCs count increased non-significantly as a result of treatment with EE or DDB or both in combination. Also, Sil treatment along with EE induced the same result. This may be an indication of the stimulation of immune system for production of leucocytes.

Plasma AST, ALT and bilirubin are the most sensitive biomarkers employed in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatocellular damage, varieties of enzymes normally located on the cytosol are released into the blood flow. Their quantification in plasma is a useful biomarker of the extent and type of hepatocellular damage (Pari and Murugan, 2004). The increased activities of these biomarkers observed in the present study correspond to the extensive hepatic damage induced in rats treated with EE. This is confirmed from histopathological signs observed in the present investigation. This was previously recorded by Venkateswaran and Pari (1997), Helal et al. (2003) and Pari and Murugan (2004), in which a marked rise of transaminases and bilirubin were recorded in EE-treated rats. Also, Harish and Shivanandappa (2005) and Biagini et al., 2006 reported elevated plasma enzyme levels as a result of induced hepatotoxicity.

Treatment with DDB and Sil induced non-significant increase of AST, ALT and bilirubin levels, suggesting that they offered protection by keeping the structural integrity of liver cell membrane against EE challenge (Pari and Murugan, 2004). These findings could be correlated with previous studies, which reported that treatment of female rats with DDB along with EE significantly reduced the levels of plasma biomarkers (Helal et al., 2003). Thus, DDB acts as a hepatoprotective agent. This phenomenon was also reported for some medicinal plants such as *Phyllanthus niruri* (Harish and Shivanandappa, 2005) and some chemicals as tetrahydrocurcumin (Pari and Murugan, 2004).

In the present study, DDB showed protection against plasma total lipids changes induced by EE, thereby denoting a wide spectrum of hepatoprotection. This result is not correlated with the findings of Helal et al. (2003), who found that DDB along with EE treatment of female rats induced a significant elevation of plasma total lipids. This contradiction might be related to sex differences. On the other hand, the present result correlated with the Livex (Venkateswaran and Pari, 1997) which showed lipid changes during hepatotoxicity. However, Sil treatment failed to reduce the total lipids significantly during EE induced hepatotoxicity.

Cholesterol level was significantly increased in rats treated with EE. This is correlated with the reduction of total lipids, which may reflect increased lipolysis rate due to enhanced lipase activity. However, DDB or Sil treatment significantly reduced the cholesterol level.

Hyperglycemia developed in rats treated with EE. DDB or Sil did not reduce the glucose level. This is an indication of pancreatitis, thyrotoxicosis and enhanced steroid activity induced by EE (Ellefson and Caraway, 1976).

EE was reported to be metabolized to reactive nitrosoalkane derivatives, which may be further metabolized to

nitroso radical. This free radical could be responsible for the degradation of phospholipids in the liver (Pessayre et al., 1985). The hepatoprotection of DDB as well as schisandrins may be due to the enhancement of liver mitochondrial GSH system, possibly through stimulation of GSH related enzymes; so that the drug may be acting by facilitation of both antioxidant and detoxification processes in the liver (Venkateswaran and Pari, 1997).

The total proteins content in the blood plasma of EE-treated rats was significantly increased, compared with the control value. This may be attributed to the hepatocellular damage and leakage of protein in the blood stream. This contradicts with those reported by Helal et al. (2003), which found non-significant increase of the total protein content in EE-treated rats. This could possibly interrelate to: (1) sex differences as reported by Helal et al. (2003) on female rats, while the present study was conducted on male rats; (2) the EE treatment in their experiment lasted only for six days, while in this study it lasted for 14 days. DDB or Sil treatment induced non-significant changes in the total proteins content, indicating hepatoprotective property.

The total albumin was significantly reduced in EE-treated rats, which is an indication of hepatic malfunctioning and decreased blood viscosity. Treatment of DDB or Sil did not restore the albumin level to the control value. The globulins level was elevated after EE treatment, which could be correlated with increased leucocytes production and reduction of albumin level. On the other hand, DDB and Sil treatment did not restore the globulins level to the control level.

EE-treated rats may potentiate focal hepatocellular damage and degeneration (Fig. 2). It is provoked by the increased yield of a highly reactive nitrosoalkane derivative, which is normally detoxified by glutathione but in excess, may consume glutathione stores, facilitating the reactive intermediate to destroy the hepatic cells and other cells (Pessayre et al., 1985). The present changes are correlated with those reported by Helal et al. (2003) and Pari and Murugan (2004). These histopathological changes were extremely reduced in rats treated with DDB and EE. Sil treated rats exhibited low evidence of histopathological signs. Therefore, the use of DDB had proved to be more efficient than Sil as a hepatoprotective drug against EE toxicity.

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