Hepatoprotective and antiobesity effects of mirabegron, a novel β3-adrenoceptor agonist, on carbon tetrachloride-induced hepatotoxicity in obese rats

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Introduction
Mirabegron is a selective β-3 receptor agonist that is used now for the management of overactive urinary bladder disease. It has potential antiobesity and antidiabetic effects. The aim of the present study was to evaluate the possible hepatoprotective and antiobesity effects of mirabegron against carbon tetrachloride (CCl4)-induced hepatic toxicity in obese rats.

Materials and methods
Five groups of animals were used in the experiment. The first group was used as a control nonobese group. The second group included obese control rats that were fed on a high-fat diet. The third group was obese rats with hepatotoxicity, which was induced by injection of CCl4. The fourth and fifth groups were obese rats with hepatotoxicity and treated with mirabegron (10 mg/kg orally) and silymarin (25 mg/kg orally), respectively. After 30 days of treatment, blood samples were used for evaluation of hepatic function markers and the liver homogenate was used for the determination of malondialdehyde and reduced glutathione levels as indicators of oxidative stress.

Results
Mirabegron 10 mg and silymarin 25 mg/kg caused a significant reduction of body weight and Lee index of obese rats. CCl4 caused abnormalities in liver enzymes with an increase in oxidative stress markers. Treatment with mirabegron and silymarin caused improvement of hepatic function parameters and a significant reduction of malondialdehyde and increase in glutathione.

Conclusion
Mirabegron has a modest antiobesity effect and is useful in the treatment of hepatotoxicity. It also has antioxidant activity, which may be responsible for its effectiveness in the treatment of CCl4-induced hepatotoxicity.

Keywords:
antiobesity, β-3 adrenoceptor, hepatotoxicity, mirabegron, oxidative stress

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Introduction

Mirabegron is a selective β-3 receptor agonist that is used now for the management of overactive urinary bladder disease [1]. The drug is not only able to control the urinary symptoms of overactive urinary bladder disease in women but also causes a significant improvement in the female sexual life [2]. It increases cAMP levels in the rat urinary bladder tissue and causes relaxation of the bladder [3]. It has a potential antiobesity effect while lacking the cardiovascular adverse effects that are associated with the classic adrenergic drugs that have a role in obesity management [4,5]. β3 adrenergic receptors are widely expressed in brown adipose tissues in addition to white adipose tissues [6].

In rodents, β-3 receptor agonist can cause an increase in energy expenditure and oxidation of fatty acid. In addition, it can improve insulin sensitivity and decrease the amount of stored fat and preserve lean body mass [5]. Stimulation of β3-adrenergic receptors in rabbits has a vascular protective role owing to the improvement in the function of endothelium and restoration of nitric oxide/redox balance [7].

Obesity is a syndrome characterized by increasing the storage of fat in the body. It results from the imbalance between intake of energy and its expenditure, and it is the major risk factor for many diseases [8,9]. Induction of a condition similar to human obesity can be done in rats by feeding on high-fat diet (HFD) that gives more than 40% energy for several weeks. Rats are more susceptible to obesity caused by diet and insulin resistance, so rats are suitable animals for the experimental method of obesity induction [10].

Carbon tetrachloride (CCl4) is a chemical that is used widely for the induction of hepatotoxicity in

animal models [11]. Free radicals are the main factors involved in the pathogenesis of hepatotoxicity caused by CCl4 as these radicals can cause lipid peroxidation of the cell membrane and damage to the cell proteins through oxidation processes [12–14]. CCl4-induced toxicity is not limited only to the liver but its toxicity may extend to affect the brain, kidney, lung, heart, and testis [15]. As mirabegron is a novel drug that lacks adequate pharmacological properties, the aim of the present study was the evaluation of the possible hepatoprotective and antiobesity effects of mirabegron against CCl4-induced hepatic toxicity in obese rats.

Materials and methods

Drugs and chemicals

Mirabegron was obtained from Astellas Pharma Europe, Netherlands (Betmiga 50 mg). Silymarin powder was obtained from SEDICO Pharmaceutical Co. (Egypt). CCl4 was purchased from Koch-Light Laboratories Ltd (England). Liver function test kits were purchased from Spectrum Diagnostics (Cairo, Egypt). The rest of the chemicals were obtained from the local commercial sources and had an analytical grade.

Animals

Thirty male Wistar Albino rats weighing 150–180 g were used in the research. Rats were purchased from the animal house of the Faculty of Medicine, Assiut University. The controlled nonobese rats consumed the ordinary laboratory food and water *ad libitum*. The research was approved by the ethics committee of the College of Medicine, University of Assiut (approval no: 17300267).

Experimental design

Induction of obesity

Obesity was induced in rats of groups 2, 3, 4, and 5 by feeding with a HFD for 7 weeks. The diet is formed of 22% mixture of sunflower oil and olive oil, 4.5% soybean, 0.5 wheat bran, 4% molasses, 68% corn starch, 0.5% common salt, and 0.5% mineral and vitamin mix. The fat content of this diet was ~ 25% [16].

Measurement of the body weight and calculation of Lee index were done at the start of the experiment, after 7 weeks, and at the end of the experiment. Any animal with an increase in body weight more than 20% was considered as an obese rat and included in the experiment [17]. The Lee index was calculated as a cubic root of body weight (g) divided by the nasoanal length (cm) multiplied by 1000 according to the modification done by Szentagothai *et al*. [18].

Induction of hepatotoxicity

Induction of hepatic toxicity was done using CCl4. It was diluted in olive oil 1: 1, v/v. CCl4 was injected intraperitoneally in a dose of 0.5 ml/kg twice weekly for 1 month [19].

Animal grouping

Five groups of animals, with six rats in each group, were used in the experiment. The first group was fed on ordinary laboratory food and injected intraperitoneally with 0.1 ml of olive oil and used as a control nonobese group. The second group was obese rats that were fed on HFD for 7 weeks and continued on feeding on HFD till the end of study and was used as control obese rats. The third group was obese rats with hepatotoxicity, which was induced after 7 weeks of feeding on HFD by injection of CCl4 (0.5 ml/kg twice weekly for 1 month). The fourth and fifth groups were obese rats with hepatotoxicity and treated with either mirabegron (10 mg/kg) or silymarin (25 mg/kg) orally using oral gavage needle for 30 days. The selection of the mirabegron and silymarin doses depends on the range of doses of previous investigations [20,21].

After the end of 30 days of treatment, decapitation of rats was done, and the blood samples were collected. Centrifugation of the blood for 10 min at 3000 revolution/min was done, and stored at −20°C till use in the assessment of liver function tests. The livers were removed and frozen in liquid nitrogen for measuring hepatic oxidative stress. Part of the liver was preserved in 10% formalin for hematoxylin and eosin staining for histopathological examination. For measuring of hepatic oxidative stress, livers were weighed, then homogenization was done in phosphate buffer. Centrifugation at −4°C for 15 min at 10 500 revolution/min was performed.

Hepatic function assessment

Measuring of the hepatic enzymes in serum, such as alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST), was done. In addition, total bilirubin and total proteins were also measured using the commercially available kits. The liver function tests were used as indicators for the hepatic function and for assessment of hepatic toxicity.

Evaluation of hepatic oxidative stress

Determination of hepatic malondialdehyde

Malondialdehyde (MDA) is a good marker for oxidative stress and lipid peroxidation. Its level was measured in rat liver homogenate by the method indicated by Ohkawa *et al*. [22]. After colorimetric reaction with thiobarbituric acid, MDA level was measured spectrophotometrically.

Determination of hepatic reduced glutathione

The hepatic glutathione (GSH) levels were measured according to the method described by Boyne and Ellman [23]. The hepatic homogenate was mixed with trichloroacetic acid 10%. Centrifugation at 5000 revolution/min was done at −4°C for 10 min. Then disodium hydrogen phosphate buffer (pH 8.4) was added to supernatant, and then Ellman's reagent was added (0.25 ml). Incubation of the samples for 10 min was done. The absorbance of the color was measured at 412 nm spectrophotometrically.

Histopathological examination

Hepatic tissue samples were cut by a special microtome (4–5 μm in thickness) and stained with hematoxylin and eosin. The sections were examined by the light digital microscopy. The damage in the hepatic tissue was scored as grade (−) if there was normal hepatic tissue, grade (+) for mild hepatic affection (1–25%), grade (++) for moderate hepatic affection (25–50%), and grade (+++) for severe hepatic affection (>50%) [24].

Statistical analysis

Data were represented as the mean±SE of six observations. To know if there was a statistical significant difference between the different groups, one-way analysis of variance was done. Tukey's post-hoc test was used for multiple comparisons. The results were considered as statistically significant differences if *P* value less than 0.05. The analysis was performed using Prism software (Graph-Pad Software, version 7, California corporation, USA).

Results

Results of the effect of different treatment on body weight and Lee index

Effect of CCl4, mirabegron (10 mg/kg), and silymarin (25 mg/kg) orally for 30 days on body weight and Lee index.

At the end of experiment, the results of body weight measurement and Lee index after feeding on the HFD for 11 weeks showed that there was a significant ($P < 0.05$) increase in the body weight and Lee index of animals fed on HFD compared with the control nonobese animals (Fig. 1 and 2). Injection of CCl4 twice weekly for 1 month caused a reduction of the body weight and Lee index of the obese animals, but this reduction was nonsignificant (Fig. 1 and 2). Treatment with mirabegron 10 mg/kg and silymarin 25 mg/kg for 30 days caused a significant ($P < 0.05$) reduction of the body weight and Lee index of the obese animals in comparison with the obese control group (Fig. 1 and 2).

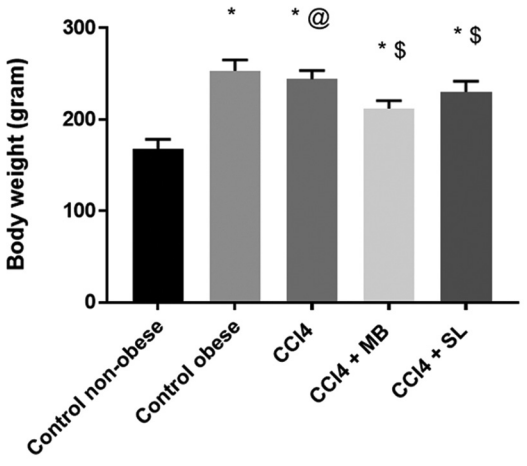
Results of liver function assessment

Effect of CCl4, mirabegron, and silymarin on serum AST, ALT, ALP, total bilirubin, and total proteins.

CCl4, when injected twice weekly for 1 month, caused severe hepatic toxicity as it was evident from Fig. 3–7, which showed a significant ($P < 0.05$) increase in serum AST, ALT, ALP, and total bilirubin, with a significant ($P < 0.05$) decrease in the total proteins.

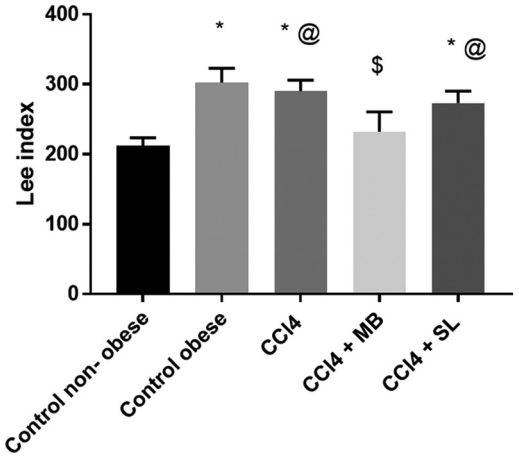
Administration of 10 mg/kg of mirabegron and 25 mg/kg of silymarin orally for 30 days resulted in a significant ($P < 0.05$) reduction in serum AST, ALT, ALP, and total bilirubin and a significant ($P < 0.05$) elevation of total proteins in comparison with the CCl4-treated group (Fig. 3–7). However, silymarin caused a more normalization of hepatic markers than mirabegron.

Figure 1



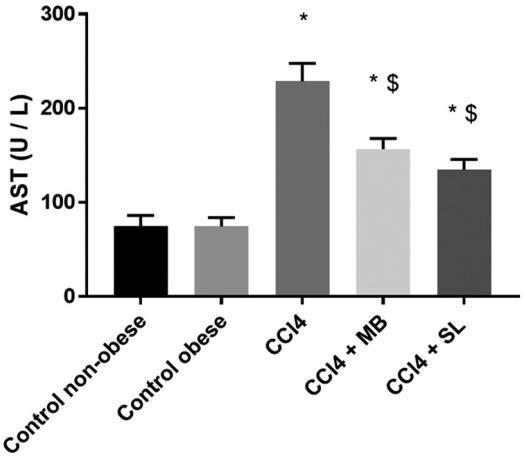
Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on rat body weight. Results were represented as mean ± SE (every group consisted of six rats). **P* value less than 0.05 in comparison with control nonobese rats. $*P* value less than 0.05 in comparison with control obese rats. @ *P* value less than 0.05 in comparison with MB 10 mg-treated rats.

Figure 2



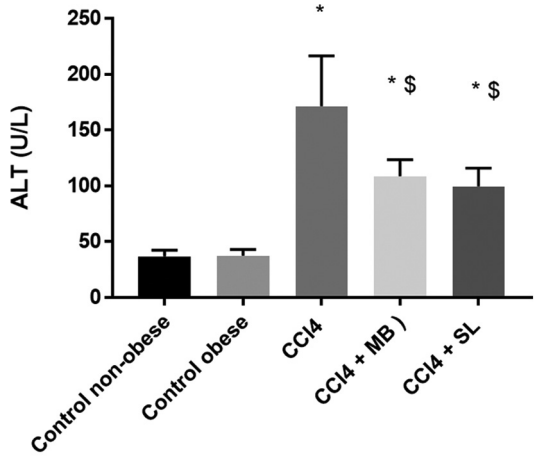
Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on rat Lee index. Results were represented as mean ± SE (every group consisted of 6 rats). **P* value less than 0.05 in comparison with control nonobese rats. $*P* value less than 0.05 in comparison with control obese rats. @ *P* value less than 0.05 in comparison with MB 10 mg-treated rats.

Figure 3



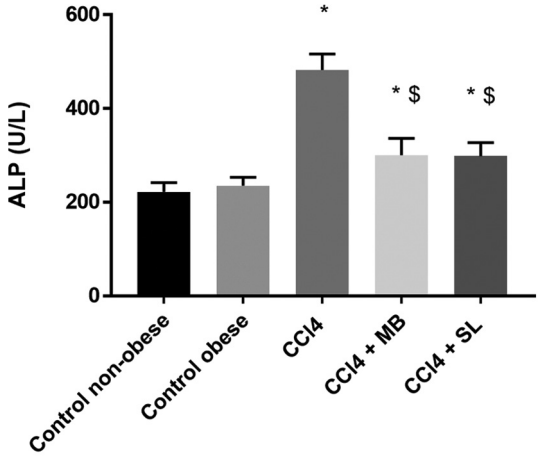
Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on rat serum aspartate transaminase (AST). Results were represented as mean ± SE (every group consisted of 6 rats). **P* value less than 0.05 in comparison with control obese rats. $*P* value less than 0.05 in comparison with CCl4-treated rats.

Figure 4



Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on rat serum alanine transaminase (ALT). Results were represented as mean ± SE (every group consisted of 6 rats). **P* value less than 0.05 in comparison with control obese rats. $*P* value less than 0.05 in comparison with CCl4-treated rats.

Figure 5



Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on rat serum alkaline phosphatase (ALP). Results were represented as mean ± SE (every group consisted of 6 rats). **P* value less than 0.05 in comparison with control obese rats. $*P* value less than 0.05 in comparison with CCl4-treated rats

Results of hepatic oxidative stress assessment

Effect of CCl4, mirabegron, and silymarin on hepatic MDA level.

CCl4, when injected for 1 month, caused a significant ($P < 0.05$) elevation of hepatic MAD in comparison with the control obese rats. Oral mirabegron 10 mg/kg and silymarin 25 mg/kg for 30 days caused a significant ($P < 0.05$) reduction in hepatic MDA (Fig. 8).

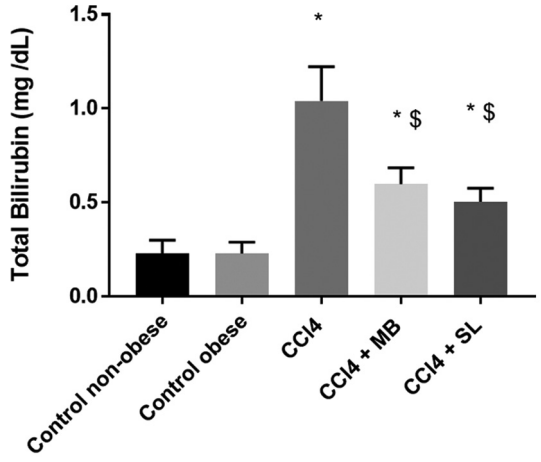
Effect of CCl4, mirabegron, and silymarin on hepatic GSH level:

The injection of CCl4 caused a significant ($P < 0.05$) reduction of hepatic GSH level. Oral mirabegron 10 mg/kg and silymarin 25 mg/kg for 30 days caused a significant ($P < 0.05$) elevation in hepatic GSH level in comparison with the CCl4-treated group (Fig. 9).

Histopathological results

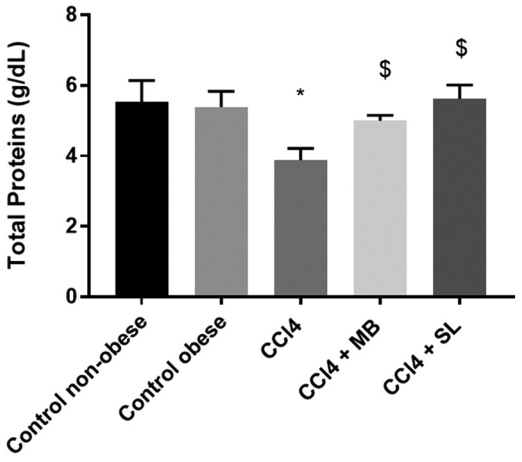
Table 1 and Fig. 10 summarize the hepatic histopathological changes that resulted from the different treatment. There was no histopathological abnormality detected in the control nonobese

Figure 6



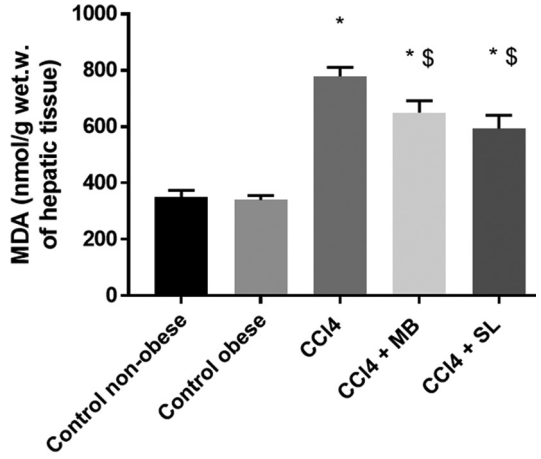
Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on total bilirubin of rat serum. Results were represented as mean ± SE (every group consisted of 6 rats). *P value less than 0.05 in comparison with control obese rats. $$P$ value less than 0.05 in comparison with CCl4-treated rats.

Figure 7



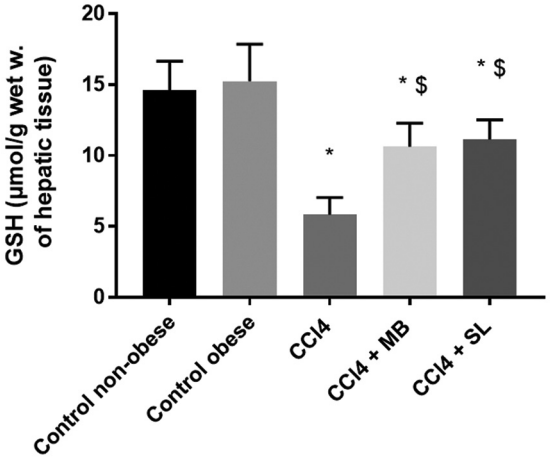
Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on total proteins of rat serum. Results were represented as mean ± SE (every group consisted of 6 rats). *P value less than 0.05 in comparison with control obese rats. $$P$ value less than 0.05 in comparison with CCl4-treated rats.

Figure 8



Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on hepatic malondialdehyde (MDA). Results were represented as mean ± SE (every group consisted of 6 rats). *P value less than 0.05 in comparison with control obese rats. $$P$ value less than 0.05 in comparison with CCl4-treated rats.

Figure 9



Effect of carbon tetrachloride (CCl4), mirabegron (MB) 10 mg/kg orally for 30 days, and silymarin (SL) 25 mg/kg orally for 30 days on hepatic reduced glutathione (GSH). Results were represented as mean ± SE (every group consisted of 6 rats). *P value less than 0.05 in comparison with control obese rats. $$P$ value less than 0.05 in comparison with CCl4-treated rats.

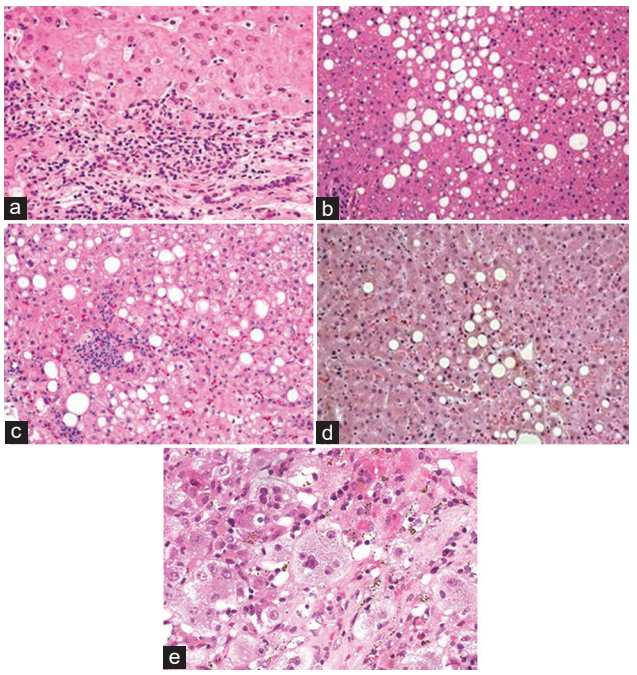
group, and the hepatic tissues appeared with normal structure (Fig. 10a). The control obese group after feeding on the HFD showed a marked fat deposition in the hepatocytes (Fig. 10b). Obese animals treated with CCl4 for 1 month showed a marked tissue necrosis, inflammatory cell infiltration in addition to fatty deposition (Fig. 10c). Treatment with mirabegron 10 mg/kg and silymarin 25 mg/kg for 1 month caused a mild improvement in the histopathological changes in comparison with the obese control group and the obese animals that were treated by CCl4 (Figures 10d and 10e).

Discussion

The results of the study indicated that the feeding of the rats with HFD caused a significant increase in the body weight and Lee index compared with the control rat group that were fed on the ordinary laboratory chew. The body weight of the rats fed on HFD was more than 20% of the initial body weight, which was in accordance with Wang *et al.* [17], who indicated that the 20% increase in body weight suggested the occurrence of obesity in rats.

Treatment with mirabegron for 30 days after the induction of obesity caused a significant reduction of

Figure 10



Histopathological changes in the hepatic tissues from the studied groups (hematoxylin and eosin, ×200). (a) Normal liver structure in the control nonobese group. (b) Marked fat deposition in the control obese group. (c) Marked hepatic necrosis with massive cellular infiltration in CCl4-treated group. (d) Mild improvement in hepatic pathology with mirabegron 10 mg treatment and (e) silymarin 25 mg treatment.

Table 1 Hepatic histopathological changes caused by feeding on high-fat diet, carbon tetrachloride, mirabegron, and silymarin

Group	Fat deposition	Inflammatory cell infiltration	Tissue necrosis
Control nonobese	–	–	–
Control obese	+++	+	–
CCl4	+++	+++	++
CCl4+mirabegron (10 mg)	+	++	+
CCl4+silymarin (25 mg)	++	++	+

The score indicated the following: no changes (–), mild hepatic changes (+), moderate hepatic changes (++), and severe hepatic changes (+++). CCl4, carbon tetrachloride.

body weight and Lee index of the obese animals, but this reduction was modest and in agreement with other observations which demonstrated that mirabegron has a mild antiobesity effect in rodents [25]. Bloom *et al.*[26] also demonstrated that CL 316 243 which is potent β-3 receptor agonist had a promising antiobesity effect. The weight-reducing effect of mirabegron may be contributed to the stimulation of β-3 adrenergic receptors in white adipose tissues that increase lipolysis and in brown adipose tissues that increase thermogenesis [27]. Stimulation of β-3 receptor in rodents increases both fatty acid oxidation and energy expenditure and improves insulin sensitivity with preservation of the lean body mass [5]. Many investigators demonstrated that β-3 receptor agonists caused a mild effect on body weight of obese animals, which was explained by the ability of these drugs to increase energy expenditure [27]. Activation of the central β3-receptors by BRL37344, the β3-adreneroceptor agonist, caused hypophagia in nonobese rat [28]. Liu *et al.*[29] reported that chronic treatment with CL-316243, which is a β3-adrenergic agonist, caused a significant reduction of the body weight, food intake, and white adipose tissues. The use of silymarin caused a mild and a significant reduction of body weight of the obese animals, but it caused a nonsignificant reduction of the Lee index. The results were in agreement with the results of Guo *et al.*[30] who demonstrated that the administration of silymarin caused loss of body weight of mice with HFD-induced obesity.

The results of this study indicated that intraperitoneally injection of CCl4 0.5 ml/kg twice weekly for one month into the rats caused a marked hepatic damage, as it was evident from the marked elevation in serum AST, ALT, ALP, and total bilirubin with a reduction in the total proteins. The results were in agreement with the results of other reports which indicated that CCl4 can cause severe hepatic damage with an elevation of hepatic enzymes and total bilirubin [31,32]. CCl4 caused elevation of serum total bilirubin, which is a marker for bile obstruction caused by hepatocyte damage [33]. Hepatotoxicity induced by CCl4 is owing to the generation of trichloromethyl free radicals, which initiate a chain of oxidative stress that activates inflammatory reactions [14].

In the present study, treatment with mirabegron for 1 month resulted in a significant decrease in the elevated hepatic enzymes and total bilirubin and a significant elevation in the serum total proteins. This was an indicator that mirabegron had a role in the treatment of hepatic toxicity, which may be owing to the result of its ability to reduce the toxic effect of CCl4 or owing to the maintenance of the normal physiological role of the liver. The reduction of the level of the elevated hepatic enzymes could be caused by healing of liver parenchyma with liver cell regeneration, which may be caused by the administration of mirabegron for 1 month. There were no sufficient data and there were limited researches regarding the role of mirabegron in treatment of hepatic toxicity.

The use of silymarin for 1 month caused a significant improvement of the elevated hepatic enzymes and improvement of the hepatic toxicity that was induced by CCl4. The results were in accordance with the report of Ayatollahi *et al.*[34] who demonstrated that silymarin inhibited the transaminase activity and prevented the progression of hepatic injury in cases of CCl4-induced hepatic toxicity. It also

enhanced the cellular regeneration and repair. Tsai *et al.*[35] reported that silymarin caused a restoration of the CCl4-induced hepatic damage, with significant reduction of the elevated serum AST, ALT, and ALP. The hepatoprotective effect of silymarin may be owing to its ability for free radical trapping and stabilization of the cytoplasmic membrane. It is highly effective in protection of the liver against poisoning by many hepatotoxic agents including CCl4 in experimental animals. Silymarin is widely used now as a liver protective agent for treatment of hepatic diseases in Asia and Europe [36].

Administration of CCl4 for 1 month caused a significant increase in the level of hepatic MDA, which is a product resulted from hepatocyte lipid peroxidation [37]. CCl4 enhances lipid peroxidation, which elevates the level of MDA that causes damaging of the hepatic tissues with the failure of the antioxidant system leading to excessive free radical production [38]. CCl4 caused also a significant reduction in the level of hepatic reduced GSH. This result is in accordance with previous reports which indicated that there was a significant depletion of GSH levels in different tissues of rat exposed to CCl4 [39]. Intracellular GSH represents the major nonenzymatic component that fights against oxidative stress [40]. Its depletion exposes tissue to oxidative injury. GSH acts as a substrate for GSH peroxidase and GSH S-transferase. It also has an important role in the detoxification of toxic products by forming GSH conjugates [41].

The treatment with mirabegron caused a significant decrease in the hepatic level of MDA with a significant increase in the GSH level suggesting that this drug had an antioxidant activity that may be responsible for the hepatoprotective effect of the drug. The antioxidant results of mirabegron were in accordance with a study that indicated that β3-adrenoceptor agonist that contained aryloxypropanolamine moiety had an antioxidant activity *in vitro* [42]. The results of the mirabegron hepatoprotective effect were also in agreement with the previous reports which demonstrated that many drugs and agents with antioxidant activity could protect against hepatotoxicity induced by CCl4 in rats [43,44].

The results indicated that the use of silymarin caused a significant improvement in the hepatic oxidative stress parameters as it reduced the elevated hepatic MDA and increased the reduced hepatic GSH. The results were in accordance with the previous studies that demonstrated that treatment with silymarin decreased the oxidative stress markers caused by HFD and caused the restoration of the activity of antioxidant enzymes in mice [45]. Nencini *et al.*[46] indicated that silymarin had antioxidant activity and could elevate the intracellular GSH in rat brain tissues. The use of silymarin in diabetic rats caused restoration of the elevated MDA and the antioxidant enzymes.

The histopathological examination showed that there was a marked fat deposition in the control obese group after feeding on a HFD. Obese animals treated with CCl4 for 1 month showed marked inflammatory cell infiltration, tissue necrosis, in addition to fatty deposition. Rats treated with mirabegron and silymarin showed attenuation of these changes with a significant improvement of CCl4-induced hepatic toxicity. The histopathological results were in agreement and confirmed the biochemical results.

Conclusion

The study showed that mirabegron had a modest antiobesity effect and antioxidant activity, which may be responsible for its effectiveness in the treatment of CCl4-induced hepatotoxicity, but its effect was less than the silymarin effect. Further studies in human are needed to confirm the potential use of mirabegron in the treatment of hepatic toxicity and obesity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Igawa Y, Michel MC. Pharmacological profile of β3-adrenoceptor agonists in clinical development for the treatment of overactive bladder syndrome. Naunyn Schmiedebergs Arch Pharmacol 2013; 386:177–183.

2 Gubbiotti M, Giannantoni A, Cantaluppi S, Coluccia AC, Ghezzi F, Serati M. The impact of Mirabegron on sexual function in women with idiopathic overactive bladder. BMC Urol 2019; 19:7.

3 Sacco E, Bientinesi R. Mirabegron: a review of recent data and its prospects in the management of overactive bladder. Ther Adv Urol 2012; 4:315–324.

4 Arch JRS. Challenges in β3-adrenoreceptor agonist drug development. Ther Adv Endocrinol Metab 2011; 2:59–64.

5 Chapple CR, Kaplan SA, Mitcheson D, Klecka J, Cummings J, Drogendijk T, *et al.* Randomized double blind, active-controlled phase 3 study to assess 12-month safety and efficacy of mirabegron, a β (3)-adrenoreceptor agonist in overactive bladder. Eur Urol 2013; 63:296–305.

6 Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nature Med 2013; 19:1252–1263.

7 Galougahi KK, Liu CC, Garcia A, Gentile C, Fry NA, Hamilton EJ, *et al.* β3 adrenergic stimulation restores nitric oxide/redox balance and enhances endothelial function in hyperglycemia. J Am Heart Assoc 2016; 5:e002824.

8 Haslam DW, James WP. Obesity. Lancet 2005; 366:1197–1209.

9 Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. Nat Rev Endocrinol 2013; 9:13–27.

10 Buettner R, Scholmerich J, Bollheimer LC. High-fat diets: modelling the metabolic disorders of human obesity in rodents. Obesity (Silver Spring)

2007; 15:798–808.

11 Parola M, Leonarduzz G, Biasi F, Albano E, Biocca ME, Poli G, *et al*. Vitamin E dietary supplementation protects against CCl4 induced chronic liver damage and cirrhosis. Hepatology 1992; 16:1014–1021.

12 Poli G. Liver damage due to free radicals. Br Med Bull 1993; 49:604–620.

13 Kaplowitz N, Aw TY, Simon FR, Stolz A. Drug-induced hepatotoxicity. Ann Intern Med 1996; 104:826–839.

14 Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003; 33:105–136.

15 Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology 2003; 62:353–356.

16 Gomaa AM, Abd El-Aziz EA. Vitamin D reduces high-fat diet induced weight gain and C-reactive protein, increases interleukin-10, and reduces CD86 and caspase-3. Pathophysiol 2017; 24:31–37.

17 Wang YM, Wang WP, Wang LP, Lü QH, Zhou XH. Calorie control increased vaspin levels of serum and periepididymal adipose tissue in diet-induced obese rats in association with serum free fatty acid and tumor necrosis factor alpha. Chin Med J 2010; 123:936–941.

18 Szentagothai J, Flerko B, Mess B, Halasz B. *Hypothalamic control of the anterior pituitary*. Budapest: Akademiai Kiado; 1962.

19 Clawson GA. Mechanisms of carbon tetrachloride hepatotoxicity. Pathol Immunopathol Res 1989; 8:104–112.

20 Sawadaa N, Nomiya M, Hood B, Koslov D, Zarifpour M, Andersson KE. Protective effect of a beta 3-adrenoceptor agonist on bladder function in a rat model of chronic bladder ischemia. Eur Urol 2013; 64:664–671.

21 Salam OM, Sleem AA, Omara EA, Hassan NS. Hepatoprotective effects of misoprostol and silymarin on carbon tetrachloride-induced hepatic damage in rats. Fundam Clin Pharmacol 2009; 23:179–188.

22 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351–358.

23 Boyne AF, Ellman GL. A methodology for analysis of tissue sulfhydryl components. Anal Biochem 1972; 46:639–653.

24 Breikaa RM, Algandaby MM, El-Demerdash E, Abdel-Naim AB. Biochanin A protects against acute carbon tetrachloride-induced hepatotoxicity in rats. Biosci Biotechnol Biochem 2013; 77:909–916.

25 Hainer V. Beta 3-adrenoceptor agonist mirabegron- a potential antiobesity drug? Expert Opin Pharmacother 2016; 17:2125–2127.

26 Bloom JD, Dutia MD, Johnson BD, Wissner A, Burns MG, Largis EE, *et al*. Disodium (R, R)-5-[2[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino] propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243). A potent beta-adrenergic agonist virtually specific for beta 3 receptors. A promising antidiabetic and antiobesity agent. J Med Chem 1992; 35:3081–3084.

27 Himms-Hagen J, Cui J, Danforth EJR, Taatjes DJ, Lang SS, Waters BL, *et al*. Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. Am J Physiol 1994; 266:R1371–R1382.

28 Kanzler SA, Januario AC, Paschoalini MA. Involvement of ß3-adrenergic receptors in the control of food intake in rats. Braz J Med Biol Res 2011; 44:1141–1147.

29 Liu X, Pérusse F, Bukowiecki LJ. Mechanisms of the antidiabetic effects of the beta 3-adrenergic agonist CL-316243 in obese Zucker-ZDF rats. Am J Physiol 1998; 274 (5 Part 2):R1212–R1219.

30 Guo Y, Wang S, Wang Y, Zhu T. Silymarin improved diet-induced liver damage and insulin resistance by decreasing inflammation in mice. Pharm Biol 2016; 54:2995–3000.

31 Patrick-Iwuanyanwu KC, Wegwu MO, Okiyi JK. Hepatoprotective effects of African locust bean and negro pepper in CCl4 induced liver damage on Wistar Albino rats. Int J Pharmacol 2010; 6:744–749.

32 Wegwu MO, Ayalogu EO, Sule OJ. Antioxidant protective effects of Cassia alata in rats exposed to carbon tetrachloride. J Appl Sci Environ 2005; 9:77–88.

33 Lin CC, Huang PC. Antioxidant and hepatoprotective effects of Acathopanax senticosus. Phytother Res 2000; 14:489–494.

34 Ayatollahi H, Omidi AA, Katebi M, Sabbagh S, Tavassolian H, Khalighi MR, *et al*. The protective therapeutic effect of Silymarin in acute hepatotoxicity of CCl4 in rats. J Gorgan Univ Med Sci 2008; 9:11–17.

35 Tsai JH, Liu JY, Wu TT, Ho PC, Huang CY, Shyu JC, *et al*. Effects of silymarin on the resolution of liver fibrosis induced by carbon tetrachloride in rats. J Viral Hepat 2008; 15:508–514.

36 Mourelle M, Muriel P, Favari L, Franco T. Prevention of CCl4 induced liver cirrhosis by silymarin. Fundam Clin Pharmacol 1989; 3:183–191.

37 Shenoy KA, Somayi SN, Bairy KL. Hepatoprotective effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats. Ind J Pharmacol 2001; 33:260–266.

38 Rotruck JT, Pope AL, Ganther H, Awanson AB, Haffeman DG, Hofekstra WG. Selenium: biochemical role as a component of glutathione peroxides. Science 1979; 179:588–590.

39 Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats. J Pineal Res 2000; 28:119–126.

40 Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983; 52:711–760.

41 Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005; 45:51–88.

42 Sichrovska L, Malik I, Sedlarova E, Csollei J, Muselik J. *In vitro* antioxidant properties of novel β3-adrenoceptor agonists bearing benzenesulfonamide Fragment. Dhaka Univ J Pharm Sci 2013; 12:23–28.

43 Goudar MA, Jaydevappa H, Mahadevan KM, Shastry RA, Habbu PV, Sayeswar HA. Isolation and characterization of secondary metabolite from habenaeria intermedia D. DON for screening hepatoprotective potential against carbon tetrachloride induced toxicity in albino rat liver. Int J Curr Pharm Res 2015; 7:57–61.

44 Afzal M, Khan R, Kazmi I, Anwar F. Hepatoprotective potential of new steroid against carbon tetrachloride-induced hepatic injury. Mol Cell Biochem 2013; 378:275–281.

45 Feng B, Meng R, Huang B, Shen S, Bi Y, Zhu D. Silymarin alleviates hepatic oxidative stress and protects against metabolic disorders in high-fat diet-fed mice. Free Radic Res 2016; 50:314–327.

46 Nencini C, Giorgi G, Micheli L. Protective effect of silymarin on oxidative stress in rat brain. Phytomedicine 2007; 14:129–135.