DRUG DISCOVERY

16(38), 2022

Hepatoprotective effects of gabapentin alone or with silymarin in carbon tetrachloride-induced hepatic damage in rats

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To Cite:
Abdel-Salam OME, Shafee N, Sleem AA. Hepatoprotective effects of gabapentin alone or with silymarin in carbon tetrachloride-induced hepatic damage in rats. *Drug Discovery*, 2022, 16(38), 124-130

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Peer-Review History
Received: 08 October 2022
Reviewed & Revised: 12/October/2022 to 16/December/2022
Accepted: 20 December 2022
Published: 24 December 2022

Peer-review
External peer-review was done through double-blind method.

DISCOVERY SCIENTIFIC SOCIETY

ABSTRACT

The aim of this study was to examine the effect of gabapentinoid drug gabapentin alone or in combination with the antioxidant silymarin on the carbon tetrachloride (CCl_4)-induced liver injury in rats. Gabapentin at doses of 25, 50, or 100 mg/kg, silymarin at 25 mg/kg, or gabapentin combined with silymarin was given once orally a day at the time of CCl_4 administration and for 2 weeks thereafter. Liver damage was assessed by determination of serum activities of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in addition to hepatic histopathology. Gabapentin given at the above doses showed significant protective effect against the hepatotoxicity caused by of CCl_4 rats, reducing serum ALT activities by 24.7%, 40.8% and 57.8%, respectively, compared to CCl_4 control group. Serum AST activities decreased by 26.5%, 41.4% and 66.1%, while ALP activities decreased by 27.7%, 38% and 58.9%, respectively. Silymarin given alone resulted in significant decrease in ALT, AST and ALP activities by 59.3%, 62.1% and 54.6%, respectively. Significant differences in ALT were observed between silymarin/gabapentin vs. their respective gabapentin (50 mg/kg) only group. Moreover, there were significant differences in AST between silymarin/gabapentin (25 and 50 mg/kg) compared with their respective gabapentin only groups. The histopathological alterations caused by CCl_4 were dose-dependently reduced after treatment with gabapentin alone or in combination with silymarin. These results indicate that that treatment with gabapentin protects against acute liver injury due to CCl_4. The study suggests a potential benefit for silymarin/gabapentin combination in treatment of liver injury.

Keywords: gabapentin, silymarin, carbon tetrachloride, liver disease

1. INTRODUCTION

Gabapentin is an anticonvulsant drug which is also used in treatment of neuropathic pain eg., post-herpetic neuralgia, diabetic neuropathy and trigeminal neuralgia as well as fibromyalgia, chronic back pain and post-surgical pain (Gilron and Flatters, 2006; Schmidt et al., 2013). Although gabapentin is a structural analogue of the inhibitory neurotransmitter γ aminobutyric acid (GABA), it does not bind to GABA receptors. Rather, it is suggested that the drug acts on the α2δ subunit of voltage-gated calcium channels in brain to inhibit neurotransmitter release at the synapse, increase descending inhibition of pain and release of inflammatory mediators (Sills, 2006; Chincholkar, 2018). Gabapentin is not metabolized in the liver and does not affect liver drug metabolizing enzymes. It is excreted solely by the kidney (Bockbrader et al., 2010). Adverse effects are related to the central nervous system and include dizziness, somnolescence and gait disturbances (Zaccara et al., 2011).

Gabapentin may be used alleviate neuropathic pain in patients with liver disease and hence the importance of investigating its effect on liver injury. In this study, the effect of gabapentin on the liver was examined in rats treated with carbon tetrachloride (CCl_4). The latter is an organic industrial solvent known to cause liver toxicity and therefore is employed in experimental animals for investigating the effect of drugs in presence of liver injury. The mechanism by which CCl_4 inflicts liver damage is mediated by its metabolite the trichloromethyl radical ($CCl_3^{\bullet}$) causing extensive lipid peroxidation and cellular perturbations (Recknagel et al., 1989; Weber et al., 2003). In addition, the possible modulation by gabapentin on the hepatic protective effect of silymarin was examined. Silymarin is a standardized extract derived from the milk thistle plant which is widely used in treatment of various inflammatory and toxic liver disorders by virtue of its antioxidant properties and was shown to protect the liver against injury caused by several toxicants (Saller et al., 2001; Gillessen et al., 2022).

2. MATERIALS AND METHODS

Animals

The study was conducted using Sprague–Dawley strain rats of both sexes (150–160 g of body weight). Rats were fed with standard laboratory chow and water ad libitum. The animal experiments were done in accordance to the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and chemicals

Carbon tertrachloride (BDH Chemicals, England) and gabapentin (Delta Pharma, Cairo, Egypt) were used in the experiments. The remaining chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and were of analytical grades. The doses of gabapentin used in the study were based upon the human dose after conversion to that of rat according to Paget and Barnes conversion tables, (1964).

Experimental groups

Rats were randomly assigned into 9 groups, each of 6 animals. Hepatic injury was induced by treating rats by gavage with CCl_4–olive oil (1:1, v/v) at a dose of 2.8 ml/kg through an orogastric tube. Rats were given one half of the dose of the initial dose of CCl_4 (1.4 mg/kg) one week after the first administration of CCl_4 in order to maintain liver injury (Abdel-Salam et al., 2012a). Starting on the first day of CCl_4 administration, rats were treated with either vehicle, silymarin, gabapentin or their combination orally once a day and for two weeks thereafter.

The following groups were studied:

Group 1: (normal control) received the vehicle (olive oil).

Group 2: Received CCl_4/olive oil and served as positive control.

Group 3: Received CCl_4/olive oil + gabapentin 25 mg/kg.

Group 4: Received CCl_4/olive oil + gabapentin 25 mg/kg + silymarin 25 mg/kg.

Group 5: Received CCl_4/olive oil + gabapentin 50 mg/kg.

Group 6: Received CCl_4/olive oil + gabapentin 50 mg/kg + silymarin 25 mg/kg.

Group 7: Received CCl_4/olive oil + gabapentin 100 mg/kg.

Group 8: Received CCl_4/olive oil + gabapentin 100 mg/kg + silymarin 25 mg/kg.

Group 9: Received CCl_4/olive oil + silymarin 25 mg/kg.

Rats were killed two weeks after drug administration by decapitation under ether anaesthesia.

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Serum liver enzymes

At the end of the study, blood samples were obtained from retro-orbital vein plexus under light ether anaesthesia. Serum AST and ALT activities were measured according to Reitman-Frankel colorimetric transaminase procedure (Crowley, 1967), whereas colorimetric determination of ALP activity was done according to the method of Belfield and Goldberg, (1971) using commercially available kits (BioDiagnostic, Egypt).

Histopathological studies

Representative liver samples were washed thoroughly with formal saline and then fixed in 10% neutral-buffered formal saline for 72 hours at least. The specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70% - 90% - 95% - absolute), cleared in xylene and then embedded in paraffin wax. Serial sections of 5 μm thick were cut and stained with Haematoxylin and eosin (Drury and Walligton, 1980) for histopathological investigation using a light microscope: Olympus Cx 41 with DP12 Olympous digital camera (Olympous optical Co. Ltd, Tokyo. Japan).

Statistical analysis

Results are expressed as mean ± SE. Statistical analysis of data was performed with the use of one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for comparing multiple groups. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used. Statistical significance was considered at a probability value of less than 0.05.

3. RESULTS

Biochemistry results

Rats treated with CCl_4 exhibited significant and marked elevations in ALT, AST and ALP serum activities which indicated the severity of hepatocellular injury and cholestasis caused by toxicant. The administration of gabapentin at doses of 25, 50 and 100 mg/kg in CCl_4-treated rats resulted in a dose-dependent decrements in serum liver activities. Serum ALT activities decreased by the above doses of gabapentin by 24.7%, 40.8% and 57.8%, respectively, compared to CCl_4 control group. There were also significant decrements in AST values by 26.5%, 41.4% and 66.1%, while ALP activities decreased by 27.7%, 38% and 58.9%, respectively, compared to CCl_4 control. Gabapentin given together with silymarin resulted in further decrements in serum liver enzymes activities compared with gabapentin treatment alone. Significant differences in ALT activities were observed between gabapentin at 50 mg/kg and silymarin/50 mg/kg gabapentin. In addition, there were significant differences in AST activities between gabapentin 25 and 50 mg/kg compared with silymarin/gabapentin (25 and 50 mg/kg) treated groups. On the other hand, the administration of silymarin to CCl_4-treated rats resulted in significantly decreases ALT, AST and ALP activities by 59.3%, 62.1% and 54.6%, respectively, compared with the respective CCl_4 control values (Table 1 & Figure 1).

Table 1 Effect of different doses of gabapentin given alone or in combination with silymarin on serum liver enzymes in CCl_4-treated rats

Treatments	Serum liver enzymes		
	ALT (U/l)	AST (U/l)	ALP (U/l)
Vehicle	49.1 ± 2.9	47.4 ± 2.3	61.3 ± 5.3
CCl_4-control	125.6 ± 5.6$^{@}$	128.2 ± 4.7$^{@}$	141.7 ± 5.8$^{@}$
CCl_4 +gabapentin 25 mg/kg	94.6 ± 3.5$^{*+@}$	94.2 ± 3.1$^{*+@}$	102.4 ± 4.9$^{*+@}$
CCl_4+ gabapentin 25 mg/kg + silymarin	72.7 ± 5.9$^{*+\#@}$	80.2 ± 2.5$^{*+@}$	92.3 ± 2.1$^{*+@}$
CCl_4 + gabapentin 50 mg/kg	74.4 ±2.3$^{*+@}$	75.1 ± 2.4$^{*+@}$	87.8 ± 2.5$^{*+@}$
CCl_4 + gabapentin 50 mg/kg + silymarin	45.8 ± 2.0$^{*+\#@}$	56.9 ± 3.0$^{*+\#@}$	70.6 ± 5.4^{*+}
CCl_4-gabapentin 100 mg/kg	53.0 ± 1.0$^{*@}$	43.5 ± 3.2*	58.2 ± 1.9*
CCl_4 + gabapentin 100 mg/kg + silymarin	42.3 ± 2.2*	41.4 ± 3.1*	51.9 ±3.6*
CCl_4 + silymarin	51.1 ± 3.9*	48.6 ± 4.5*	64.4 ± 4.9*

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase. Data are presented as mean ± SEM (n = 6). @p<0.05 *vs.* vehicle, *p<0.05 *vs.* CCl_4 control group, +p<0.05 *vs.* silymarin, #p<0.05 gabapentin/silymarin combination *vs.* the preceding CCl_4 + gabapentin group (without silymarin)

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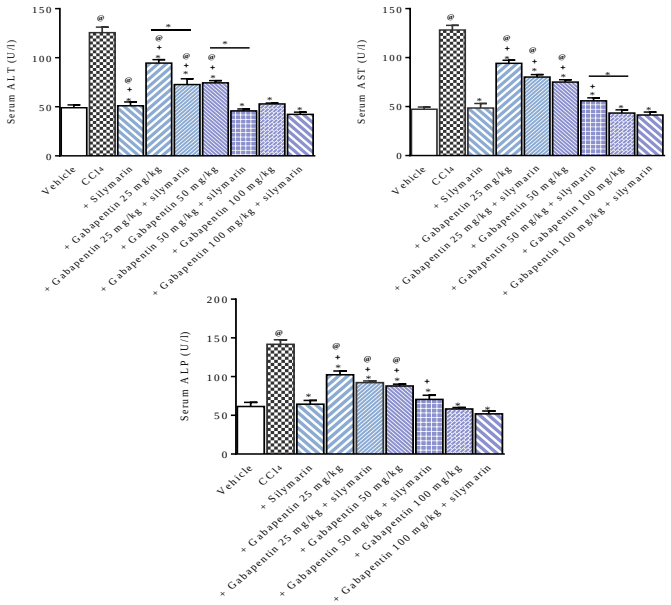

Figure 1 Effect of gabapentin alone or combined with silymarin on serum liver enzymes in CCl_4-treated rats. Data are presented as mean ± SEM (n = 6). @p<0.05 *vs.* vehicle, *p<0.05 *vs.* CCl_4 control group and between different groups as indicated in the graph. +p<0.05 *vs.* silymarin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase.

Histopathological results

The liver of vehicle-treated group showed the normal hepatic architecture (Figure 2A). The administration of CCl_4 caused marked liver tissue damage in the form of distortion of the normal architecture of liver tissue, massive fibrosis around central vein with cellular infiltration, dilatation of blood sinusoids and vacuolar degeneration in hepatocytes (Figure 2B). The co-administration of CCl_4/silymarin was associated with mild amelioration of liver tissue as it decreased fibrosis and dilatation of blood sinusoids, while vacuolar degeneration of hepatocytes was still present (Figure 2C). Gabapentin given to CCl_4-treated rats caused dose-dependant amelioration of the hepatotoxic effect of CCl_4. The least protective effect was observed with the gabapentin at 25 mg/kg. The protective effect increased with increasing the dose of gabapentin. This hepatoprotective effect was augmented by using silymarin with gabapentin (Figure 3).

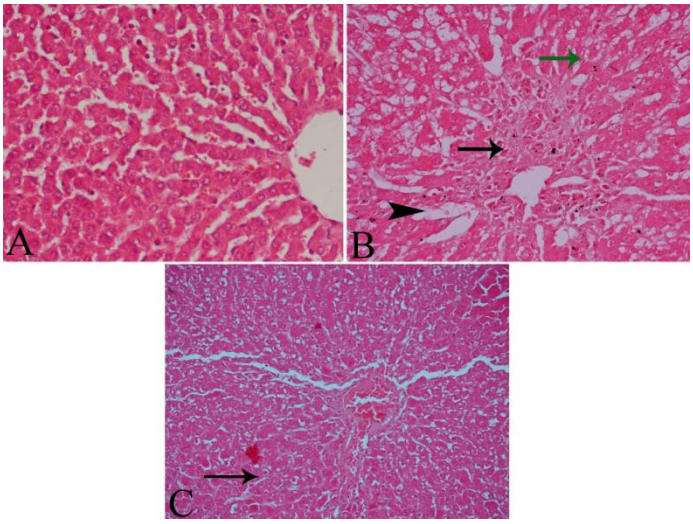

Figure 2 Representative photomicrographs of liver sections after treatment with; (A) Vehicle: Showing normal liver tissue; (B) CCl_4 control shows distortion of the normal architecture of liver tissue, massive fibrosis around central vein with cellular infiltration (arrow), dilatation of blood sinusoids (arrowhead) and vacuolar degeneration in many hepatocytes (green arrow); (C) CCl_4 + silymarin shows decrease of fibrosis around main blood vessel (arrow). No dilatation of blood sinusoids. Vacuolar degeneration of hepatocytes is still observed but with less severity than that in previous group.

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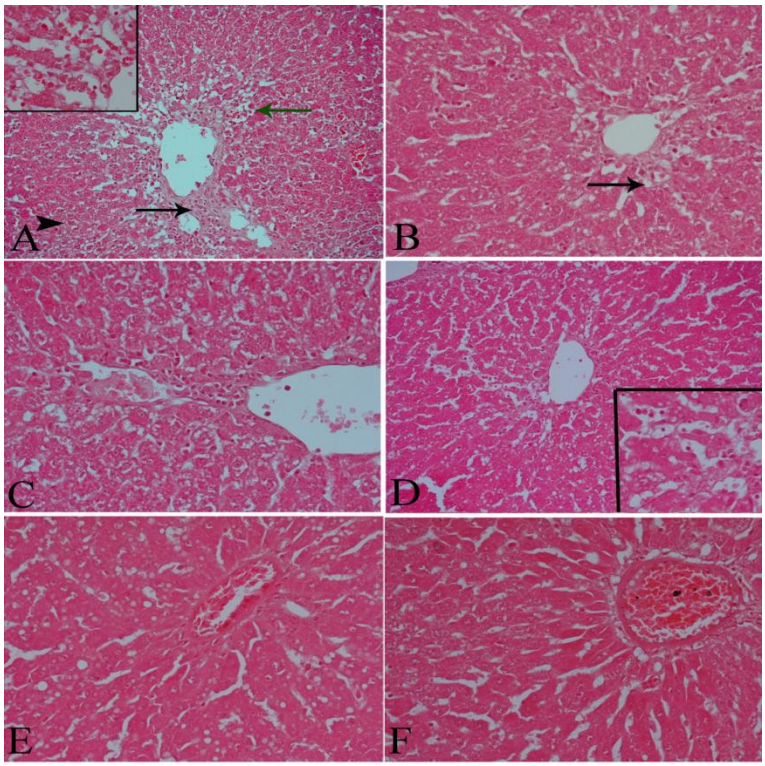

Figure 3 Representative photomicrographs of liver sections after treatment with; (A) CCl_4 + gabapentin 25 mg/kg shows dilatation of main blood vessels, massive fibrosis with cellular infiltration (arrow). Many hepatocytes show vacuolar degeneration especially in the peripheral area of the lobule (arrowhead). Noticeable dilatation of blood sinusoids is observed (green arrow). (B) CCl_4 + gabapentin 25 mg/kg + silymarin shows marked decrease of fibrosis and cellular infiltration, although dilatation and congestion of blood sinusoids is still present (arrow); (C) CCl_4 + gabapentin 50 mg/kg shows marked dilation of main blood vessels and blood sinusoids. Many hepatocytes show vacuolar degeneration but with no fibrosis; (D) CCl_4 + gabapentin 50 mg/kg + silymarin shows no fibrosis, mild dilatation of blood sinusoids with noticeable decrease of cellular infiltration and vacuolar degeneration; (E) CCl_4 + 100 mg/kg gabapentin shows no fibrosis, no cellular infiltrate, very mild vacuolar degeneration and dilatation of blood sinusoids. Thickening of main blood vessel wall is observed; (F): CCl_4 + 100 mg/kg gabapentin + silymarin shows marked amelioration as all items of damage seen in previous groups are absent.

4. DISCUSSION

The findings in the present study indicated for the first time that gabapentin exerts hepatic protective effects against acute liver injury induced by CCl_4 in the rat. Gabapentin treatment led to a dose-dependent decrease in serum activities of the liver enzymes. Serum alanine aminotransferase (ALT) activity is considered a major biochemical marker of hepatocyte damage. Aspartate aminotransferase (AST) activity in serum is also a widely used marker of liver cell injury. These enzymes are released by damaged hepatocytes into the extracellular space and consequently gain access to the circulation and reflect hepatocellular damage. On the other hand, elevated serum alkaline phosphatase (ALP) injury often reflects a cholestatic injury from damage to bile ducts or alterations in bile flow (Giannini et al., 2005; Yang et al., 2014). The protective effect of gabapentin is supported by its ability to decrease the extent of histologic liver damage.

The liver being the major site for detoxification and elimination of xenobiotics, drugs and environmental toxin is subject to damage by these chemicals and their metabolites (Österreicher, 2012). Liver injury caused by CCl_4 is free radical based process. Metabolic activation of this organic solvent by cytochrome P450-dependent monooxygenases results in the production of its reactive metabolites trichloromethyl ($CCl_3^{\bullet}$) and trichloromethyl peroxyl ($CCl_3O_2^{\bullet}$) radicals within the membrane of the endoplasmic reticulum. These highly reactive species bind covalently to the cellular membrane lipids and cause extensive lipid peroxidation in mitochondria and endoplasmic reticulum, with eventual cellular damage (Recknagel et al., 1989; Weber et al., 2003).

This fundamental role of oxidative stress in mediating the CCl_4-induced hepatic cellular damage is supported by the ability of antioxidants to exert hepatic protection, suggesting that they interfere with the underlying pathogenetic process (Naziroğlu et al., 1999; Tsai et al., 2008; Knockaert et al., 2012; Abdel-Salam et al., 2015). The mechanism by which gabaepetin exerts its protective

effect on liver injury is not clear but the drug obviously affects pathways by which CCl_4 inflicts its liver toxicity. The drug has been shown to reduce lipid peroxidation and restore glutathione levels in diabetic rat retinas (Ola et al., 2019). In contrast, gabepentin was found to increase lipid peroxidation and impair antioxidants in brain of rats with chemically-induced demyelination (Abdel-Salam et al., 2012b). The drug was shown to exert anti-inflammatory effects (Abdel-Salam and Sleem, 2009). Other mechanisms eg., an effect on inflammatory mediators, may thus be involved in gabapentin's hepatic protective effect.

Our results also that the combined treatment with silymarin and gabapentin caused noticeable decrease of serum liver enzymes activities, vacuolar degeneration, cellular infiltration and fibrosis, suggesting an additive effect. Silymarin is a flavonolignan from the seeds and fruit of *Silybum marianum* containing silibinin, silydianine and silychristine. The most active constituent is silibinin (Fraschini et al., 2002). Silymarin was shown to be an efficient hepatic protective in several experimental liver toxicity models including CCl_4 via an antioxidant and free radical scavenging and cell membrane stabilizing properties. Silymarin also exerts an inhibitory effect on stellate cell activation and consequently reduces liver fibrosis (Fraschini et al., 2002). Silymarin may be useful adjunct or supportive therapeutic option in patients during the early stage's toxic liver disease in view of its ability to combat oxidative stress, the main mechanism that mediates the toxicants-induced hepatocellular damage (Gillessen et al., 2022).

5. CONCLUSIONS

The results of the present study suggest a protective effect for the gabapentinoid drug gabapentin in the model of acute liver injury caused by CCl_4. Gabapentin can be used safely with silymarin in patients with liver disease.

Conflict of interest

The authors declare that there are no conflicts of interests

Ethical approval

The animal experiments were done in accordance to the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Funding

This study was not supported by research grants

Author contribution

O.M.E.A.S. and A.A.S. conducted the research and biochemical studies, N.S. performed the histopathology and its interpretation, O.M.E.A.S. wrote and prepared the manuscript, O.M.E.A.S, and A.A.S. and N.S. approved the final version of the manuscript.

Data and materials availability

All data associated with this study are present in the paper

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