Hepatoprotective effect of the N-alkylated isobornylamine against CCl4-induced chronic liver damage in mice

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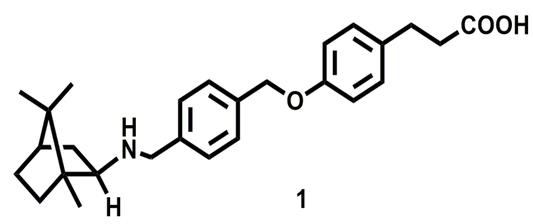
Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease caused by impaired lipid and carbohydrate metabolism. There are currently no approved drugs for the treatment of NAFLD, so their search remains an urgent task for present pharmacology. Previously, N-alkylated isobornylamine (compound **1**) at a dose of 30 mg/kg was shown to resolve of liver fatty degeneration in mice with the type 2 diabetes mellitus (C57Bl/6^{Ay}), which improved their glucose tolerance [1]. In this work, we conducted an investigation of the hepatoprotective effect of compound **1** on carbon tetrachloride (CCl_4) – induced liver injury.



N-alkylated isobornylamine

Materials & methods

Male CD-1 mice weighting 25-30 g were divided into 7 groups.

Group	Test compounds
1 «Intact control»	Vehicle (distilled water + 2 drops Tween 80)
2 «Negative control»	Vehicle + CCl_4
3 «Positive control»	Silymarin 100 mg/kg (Legalon 70, MADAUS, GmbH)
4	Compound **1** 60 mg/kg + CCl_4
5	Compound **1** 90 mg/kg + CCl_4
6	Compound **1** 120 mg/kg +CCl_4
7	Compound **1** 150 mg/kg + CCl_4

The animals were treated daily *per os* with the test compound for 3 weeks. Groups 2-7 were administered *per os* with a 0.5% CCl_4 solution in olive oil twice a week one hour after the test compound. At the same time, this groups received 5% ethyl alcohol solution instead of water *ad libitum* throughout the experiment.

Results

Blood biochemical parameters of CD-1 mice treated by Compound 1

Group	ALT, U/L	AST, U/L	ALKP, U/L	TP, g/dL
1	15.29±1.28*	41.49±3.26*	40.96±5.63*	85.30±0.84
2	26.5±1.68	52.06±2.56	68.01±5.03	85.76±0.74
3	20.75±1.35*	39.59±2.71*	43.08±3.57*	83.7±1.00
4	21.07±1.95*	44.48±3.53	82.71±14.25	84.23±1.69
5	25.82±1.72	52.93±4.41	75.62±4.73	85.05±1.43
6	20.97±1.39*	40.83±3.34*	60.44±6.02	84.98±0.75
7	14.95±1.36*,#	45.78±6.93	54.35±6.33	83.19±1.18

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; TP, total protein; *p<0.05 as compared with negative control; #p<0.05 as compared with positive control

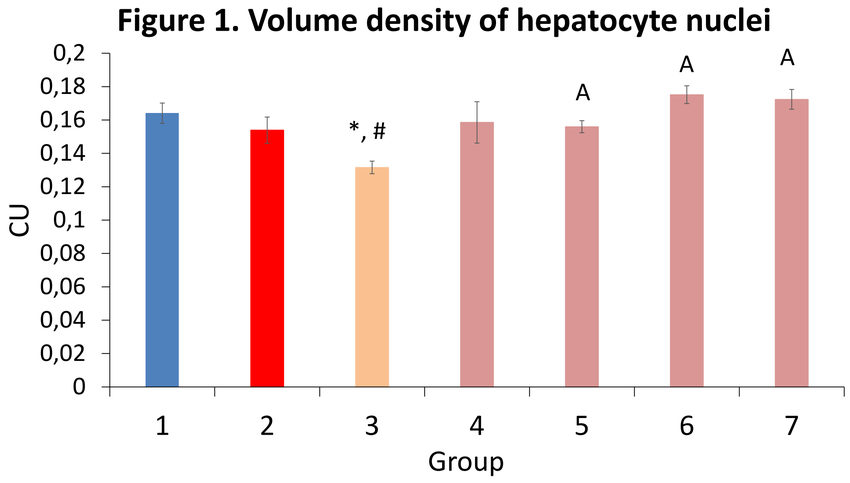
*The introduction of **1** dose-dependently reduced the concentration of ALT and ALKP in the mice's blood, and a more pronounced effect was observed at a dose of 120 and 150 mg/kg. A decrease in the concentration of AST was also noted after introduction of **1** at a dose of 120 mg/kg, similarly to the mice in the positive control group.*

Results

Relative volume of nuclei and cytoplasm of hepatocytes, sinusoids and liver necrosis

The relative volume was calculated to quantify the ability of **1** to restore the state of the mouse liver after exposure to CCl4. The greater the volume density of the nuclei and cytoplasm of hepatocytes, sinusoids, and the lower the density of liver necrosis, the better the hepatoprotective effect of the test compound.

Figure 1. Volume density of hepatocyte nuclei

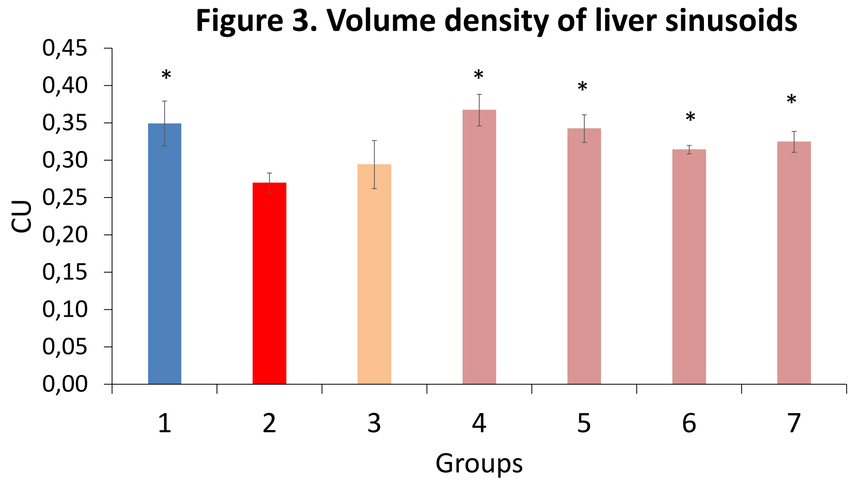


*p<0.05 as compared with negative control; #p<0.05 as compared with intact control; Ap<0.05 as compared with Silymarin 100 mg/kg; CU, conventional units.

Figure 2. Volume density of hepatocyte cytoplasm

*p<0.05 as compared with negative control; #p<0.05 as compared with intact control; Ap<0.05 as compared with Silymarin 100 mg/kg; CU, conventional units.

Figure 3. Volume density of liver sinusoids



*p<0.05 as compared with negative control; CU, conventional units.

Figure 4. Bulk density of liver necrosis

*p<0.05 as compared with negative control; #p<0.05 as compared with intact control; Ap<0.05 as compared with Silymarin 100 mg/kg; CU, conventional units.

Prolonged administration of a 0.5% CCl_4 solution was found to have no affect on the volume density of the nuclei and cytoplasm of hepatocytes except mice in positive control group (Fig. 1, 2). With the introduction of silymarin, a decrease in the volume density of nuclei and an increase in the cytoplasm of hepatocytes were observed in comparison with intact and negative controls (Fig. 1, 2).

The volume density of the liver sinusoids after the administration of silymarin remained at the level of the negative control (Fig. 3). An increase in the volume density of the liver sinusoids, on the contrary, was observed in the experimental groups (Fig. 3).

*A decrease in the volume density of liver necrosis is one of the main indicators of the hepatoprotective effect, which can be observed with the introduction of silymarin at a dose of 100 mg/kg and **1** at all doses studied (Fig. 4).*

Conclusion

The compound 1 at doses of 120 and 150 mg/kg was found to improve mice's liver condition during chronic CCl4 induced liver damage. Thus, isobornylamine derivative exhibits a hepatoprotective effect not only in metabolic liver injury, but also in toxic damage by carbon tetrachloride.

References:

1. Kuranov S, Luzina O, Khvostov M, et al. Bornyl derivatives of p-(Benzyloxy)phenylpropionic acid: In vivo evaluation of antidiabetic activity. Pharmaceuticals. 2020;13(11):1-22. doi:10.3390/PH13110404

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