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Therapeutic effects of N-acetyl-L-cysteine on liver damage induced by long-term CCl_4 administration

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Abstract. N-acetyl-L-cysteine (NAC) is a drug routinely used in several health problems, e.g. liver damage. There is some information emerged on its negative effects in certain situations. The aim of our study was to examine its ability to influence liver damage induced by long-term burden. We induced liver damage by CCl_4 (10 weeks) and monitored the impact of parallel NAC administration (daily 150 mg/kg of b.w.) on liver morphology and some biochemical parameters (triacylglycerols, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, bile acids, proteins, albumins and cholinesterase). NAC significantly decreased levels of bile acids and bilirubin in plasma and triacylglycerols in liver, all of them elevated by impairment with CCl_4. Reduction of cholesterol induced by CCl_4 was completely recovered in the presence of NAC as indicated by its elevation to control levels. NAC administration did not improve the histological parameters. Together with protective effects of NAC, we found also its deleterious properties: parallel administration of CCl_4 and NAC increased triacylglycerols, ALT and AST activity and significantly increased plasma cholinesterase activity. We have observed nonsignificantly increased percentage of liver tissue fibrosis. Our results have shown that NAC administered simultaneously with liver damaging agent CCl_4, exhibits not only protective, but also deleterious effects as indicated by several biochemical parameters.

Key words: CCl_4-induced liver damage — N-acetyl-L-cysteine — Histological parameters — Biochemical parameters

Introduction

N-acetyl-L-cysteine (NAC) is a drug that was first reported to have clinical benefits in the early 1960s, when it was shown to be an effective mucolytic agent. Later on, its contribution to the treatment of many pathologies has been described, for example: chronic obstructive pulmonary disease, bronchitis, AIDS, ischemic reperfusion injury of the heart (de Andrade et al. 2015) and acute liver failure (Kortsalioudaki et al. 2008). It has been also used in the treatment of poisoning (de Andrade et al. 2015). In clinical trials, after intoxication with acetaminophen, NAC administration was highly effective in preventing hepatocellular necrosis and confirmed its antidote function through induction of glutathione synthesis (Kortsalioudaki et al. 2008). The positive effect of NAC is related to increased antioxidant ability as it is the exogenous source of cysteine, a precursor of glutathione synthesis.

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NAC has also ability to directly scavenge reactive oxygen and nitrogen species (Lai et al. 2012) and there are other yet unknown mechanisms (Lai et al. 2012) by which NAC can act. Complete and detailed mechanisms of its action need to be discovered (de Andrade et al. 2015).

Although in practice NAC is considered to be safe, sporadically new information appears about situations/cases in which NAC not only does not help, but its administration is associated with deterioration in the body. Several authors report that NAC has the potential to act as a pro-oxidant; therefore it has been suggested to avoid its administration in the absence of a significant oxidative stress (Samumi et al. 2013). In patients with multi-system organ failure (Molnar et al. 1999), NAC showed no benefit and in fact was noted to be harmful if given 24 h after admission to the intensive care unit.

There are interesting observations of NAC effects after the intoxication with paracetamol, where about 10% of patients after the administration of NAC had anaphylactoid reaction. Return response analysis showed that these negative reactions were in cases where the level of paracetamol was relatively low – which means that the oxidative stress was minimal (Samumi et al. 2013).

NAC given to healthy volunteers in daily dose of 1.2 g for weeks followed by 2.4 g *per* day for 2 weeks increased oxidative stress, as determined by the ratio of reduced to oxidized glutathione in the whole blood. This is probably caused by auto-oxidation of NAC, an unwanted effect often observed with thiol-containing compounds (Kleinveld et al. 1992).

Liver damage is a growing problem worldwide. Therefore, it is necessary to pay great attention to study of various stages of liver damage and therapeutically interfere with these processes. One of the many years' proven animal models of liver damage is an administration of carbon tetrachloride (CCl_4).

Carbon tetrachloride toxicity mechanism has been studied since the 50's of the last century resulting in the widely accepted view that CCl_4 hepatotoxicity depends on the reductive dehalogenation of CCl_4 catalysed by cytochrome P-450 in the endoplasmic reticulum of liver cells. It has also become clear that a cascade of secondary mechanisms is evoked by the initial events of CCl_4 metabolism, and that the secondary mechanisms are responsible for ultimate plasma membrane disruption and other effects leading to death of the cell (Recknagel et al. 1989).

Experimentally induced cirrhotic response in the rat by CCl_4 is shown to be similar to human cirrhosis of the liver (Tamayo 1983; Natarajan et al 2006).

Whereas, there are some references in the literature reporting negative effects of N-acetylcysteine on the body, we decided to analyse both its therapeutic effect on liver damaged by the long-term administration of CCl_4 as well as possible additional harmful effects on this tissue.

Materials and Methods

Animals and experimental protocol

The experimental procedures used in the present study were carried out according to guidelines for the care and use of experimental animals and were approved by The State Veterinary and Food Administration of the Slovak Republic (No. Ro 1766/12-221/3). All experiments were performed in accordance with EU (2010/63/EEA).

32 adult male Wistar rats (357.3 ± 23.9 g body weight) were enrolled in this study. The animals were fed the standard Larsen diet. All animals were allowed free access to tap water and pellet diet and were maintained at temperature 22 ± 2°C, humidity 50%, under 12 h light/dark cycle. Rats were kept in groups of four to six in polypropylene cages. They were held in quarantine (1 week) and then they were randomly assigned to the Control ($n = 16$) and Carbon tetrachloride (CCl_4; $n = 16$) experimental groups. CCl_4 was injected *intraperitoneally* as a 50% (v/v) solution in olive oil (1 ml/kg) (CCl_4 group) or rats were injected only by vehicle – olive oil (Control group) twice a week for 10 weeks. Each experimental group was divided into two subgroups – without intervention (Control and CCl_4 groups) and subgroups receiving daily NAC (150 mg/kg in aqueous solution) orally by gastric tube (Control+NAC and CCl_4+NAC groups). Groups without intervention (Control and CCl_4) received daily water by the same way.

Ten weeks after CCl_4 administration and 48 h after the last treatment with CCl_4 the rats were anesthetized with thiopental (60 mg/kg), blood samples were collected and liver tissue was taken for histopathological analyses and preparation of homogenate.

Liver histology

Liver samples were fixed in 4% formaldehyde and embedded in paraffin. They were cut in 4 μm slices (Leica RM 2135, Germany) and stained routinely with hematoxylin and eosin. Modified picrosirius red staining technique (Dolber et al. 1993) was used to determine the level of fibrosis. Briefly, after deparaffinization in xylene and rehydration in distilled water, the slides were incubated in 0.2% aqueous solution of phosphomolybdic acid and stained with 0.1% sirius red in saturated solution of picric acid for 90 min. Finally, the slides were washed in 0.01 M hydrochloric acid. Total fibrosis of each tissue sample was analysed by light microscopy in polarized light (Microscope Leica DM2000 (Wetzlar, Germany)). The macrovesicular steatosis was analysed by light microscopy in hematoxylin and eosin stained slides. The represented fibrotic resp. steatotic area was measured by histomorphometry in 10 microscopic fields at 20× magnification using the ImageJ morphometric software v.1.51 (National Institutes of Health, USA) (Abramoff et al. 2004).

Results are expressed as the steatotic area out of the total analysed area, compared to controls (100%).

Blood collection

Blood was collected from the abdominal aorta of anaesthetized rats into heparin coated tubes, centrifuged (1200 × *g*, 10 minutes) to separate plasma from the blood elements. Plasma samples were immediately aliquoted and stored at −80°C until biochemical analysis.

Liver homogenate

The liver tissue (1 g) was gently homogenized in 10 ml cold sucrose solution (0.25 M) + 1 mM EDTA (pH 7.4), using a tissue glass Teflon Potter homogenizer 1 time for 25 s. The liver homogenate was used for analyses of total proteins and TAG.

Samples of liver tissue for cholesterol determination were homogenized in a chloroform-methanol mixture (1:1).

Biochemical analysis

Concentrations of albumin (Alb), total proteins (tProt), total cholesterol (tChol), triacylglycerols (TAG), bilirubin (tBil) and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) in plasma were determined in an accredited laboratory (AlphaMedical s.r.o, Slovakia) using a ADVIA 1800 Chemistry System (Siemens Healthcare Diagnostics, Germany). Activity of cholinesterase (CHE) in plasma was determined with photometric method according to Knedel and Bottger (1967). Bile acids were determined with photometric method according to Nicolas et al. (1980) and Mashige et al. (1981).

Total proteins in liver homogenate were determined according to Lowry (1951), cholesterol according to Abell et al. (1952). Triacylglycerols were determined by the commercial diagnostic kit DOT Diagnostics s.r.o, Czech Republic, Triglyceridy DST-P.

Statistical analysis

The experimental data were expressed as the mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by LSD *post hoc* test for multiple comparisons (IBM SPSS Statistics 24, USA). The limit for statistical significance was set at $p < 0.05$.

Results

Weight differences and hepatosomatic index

The health status of the rats was evaluated in term of weight gain and the liver status in term of the ratio of liver weight to body weight (hepatosomatic index – HI) at the end of the experiment (Table 1). CCl_4 induced significant changes of both parameters: weight gain was decreased and hepatosomatic index was increased. Treatment with NAC resulted in the decrease of weight gain in Control+NAC and CCl_4+NAC groups, but only in the Control+NAC group the decrease was significant. HI was not affected by NAC treatment. We have also found a statistically significant difference between Control+NAC and CCl_4+NAC groups in both parameters.

Histological parameters

Administration of CCl_4 led to the development of liver steatosis and fibrosis and when compared to controls significantly increased steatotic areas (584.6 ± 64.16%) and collagen level (737.9 ± 64.07%) in liver. The co-treatment with NAC had no significant impact resp. benefit when compared to the Control resp. CCl_4-exposed rats (Table 2, Figs. 1–4).

Table 1. Body weight differences and hepatosomatic index at the end of the experiment

	Control	Control+NAC	CCl_4	CCl_4+NAC
Difference of body weight (g)	96.75 ± 5.30	61.25 ± 7.73*	13.00 ± 21.25**	$12.44 \pm 6.28^{++}$
Hepatosomatic index (g/g)	0.030 ± 0.0006	0.028 ± 0.0003	0.039 ± 0.0029**	$0.039 \pm 0.0016^{++}$

Data are presented as the mean ± SEM ($n = 8$); * $p < 0.05$; ** $p < 0.01$ *vs.* Control group; $^{++}$ $p < 0.01$ *vs.* Control+NAC group.

Table 2. Histological parameters

	Control	Control+NAC	CCl_4	CCl_4+NAC
Steatosis (%)	100 ± 15.57	109.8 ± 44.44	$584.6 \pm 64.16^{***}$	$420.2 \pm 62.55^{++}$
Fibrosis/collagen level (%)	100 ± 9.936	129.5 ± 17.14	$737.9 \pm 64.07^{***}$	$886.5 \pm 66.7^{++}$

Data are presented as the mean ± SEM ($n = 8$); *** $p < 0.001$ *vs.* Control group; $^{++}$ $p < 0.01$ *vs.* Control+NAC group.

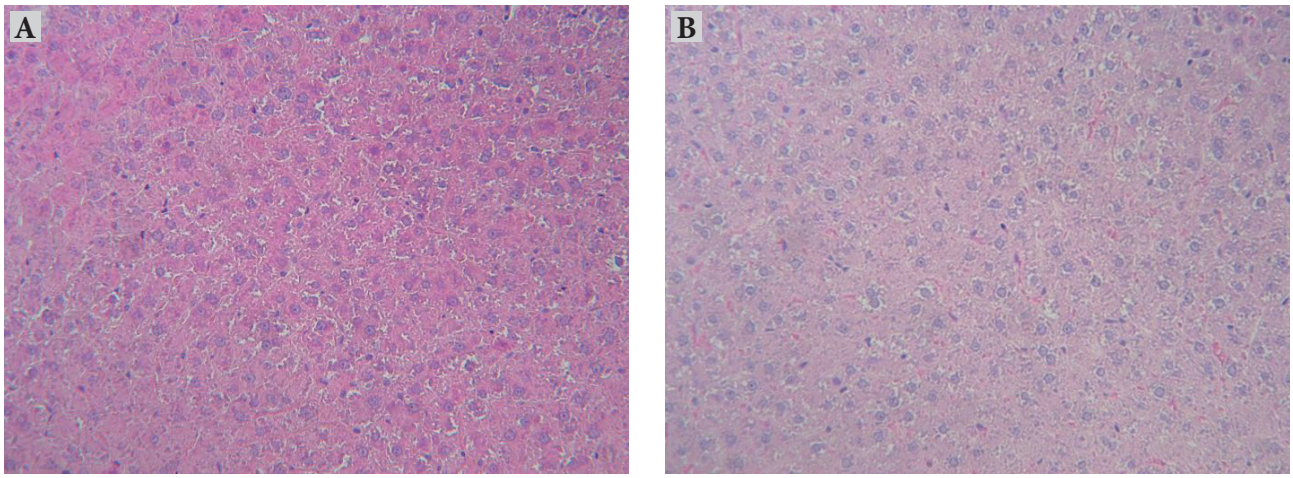

Figure 1. Histopathological picture of steatosis in the Control group (**A**) and Control+NAC group (**B**) (H&E, magnification ×200).

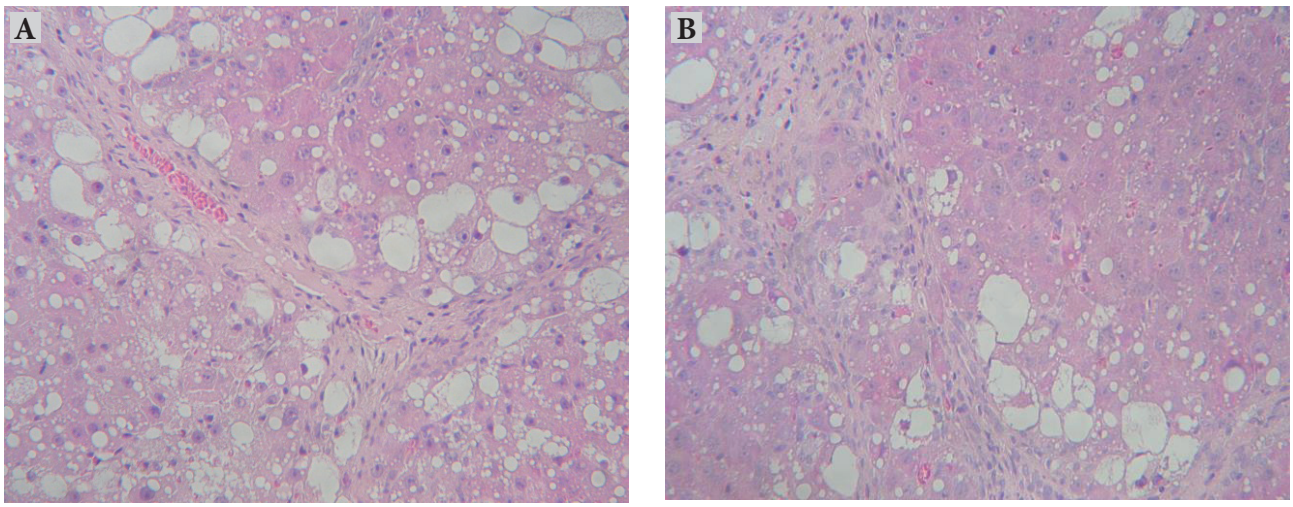

Figure 2. Histopathological picture of steatosis in the CCl_4 group (**A**) and CCl_4+NAC group (**B**) (H&E, magnification ×200).

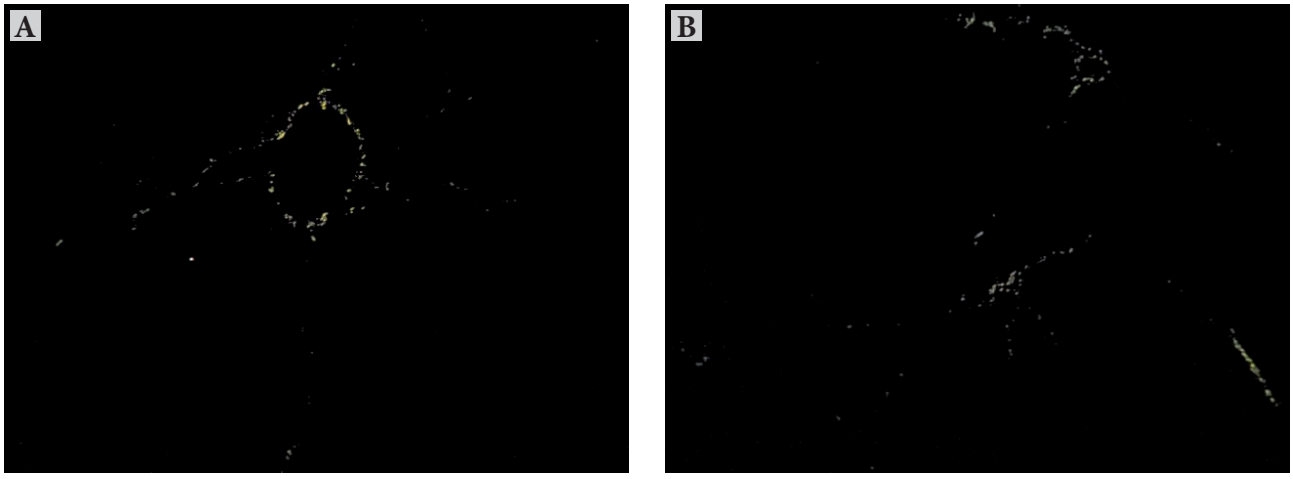

Figure 3. Histopathological picture of fibrosis in the Control group (**A**) and Control +NAC group (**B**) (magnification ×200).

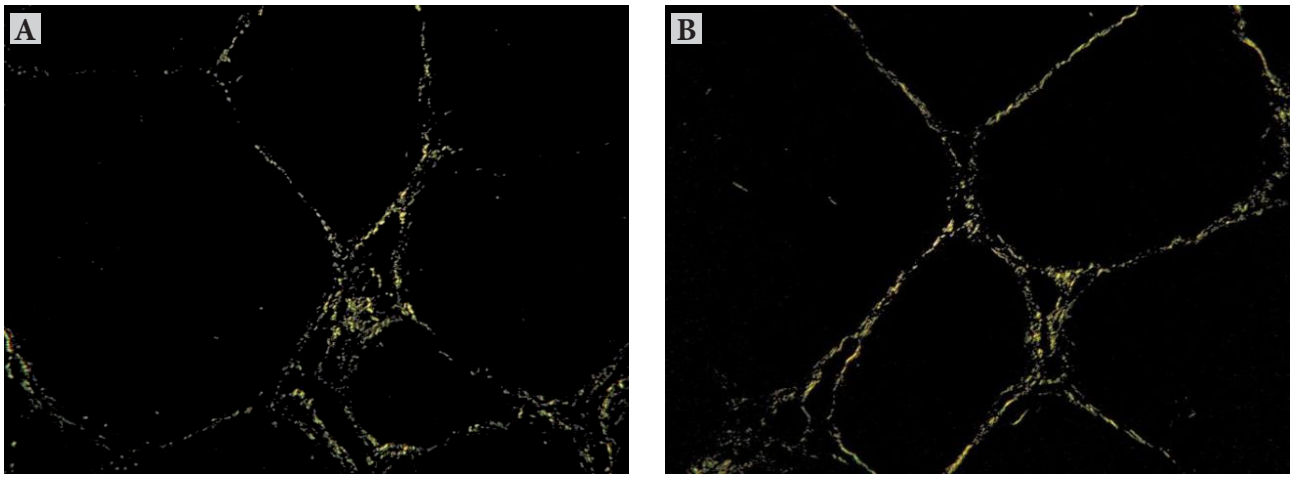

Figure 4. Histopathological picture of fibrosis in the CCl_4 group (**A**) and CCl_4+NAC group (**B**) (×200).

Total proteins, cholesterol and triacylglycerols in liver

Induction of liver damage by CCl_4 decreased tProt level and increased tChol and TAG concentrations in liver tissue in comparison to the Control group. NAC administration significantly decreased concentration of TAG in liver of CCl_4+NAC rats. Concentration of TAG decreased by 46.25% in the CCl_4+NAC group compared to CCl_4 group. However, achieved value still represents an increase by 145.34% compared to the Control, or by 205.31% compared to the Control+NAC (Table 3).

Blood plasma parameters of liver function

CCl_4-induced liver damage resulted in significantly increased parameters of hepatocellular damage in plasma (activities of ALT, AST, concentration of tBil, tBile acids and activity of CHE) and significantly decreased concentration of tProt, albumin, tChol and TAG in the CCl_4-exposed rats compared to the Control rats. Some parameters (TAG (p = 0.07), AST (p = 0.07) and CHE (p = 0.02)) were after co-treatment with NAC increased compared to the CCl_4 group (Table 4). The treatment with NAC significantly decreased concentration of tBil and tBile acids, but values did not reach the level of the Control group, resp. Control+NAC group. Only tChol, was increased and reached the level of the Control resp. Control+NAC group after the treatment with NAC. Significant difference between groups Control+NAC and CCl_4+NAC was observed in parameters such as ALT, AST activities, tBile acids and CHE.

Impact of NAC to the Control group

NAC administration to Control group of rats did not have any significant effect to studied parameters (Table 1–4) except of difference of body weight, where was observed reduction in comparison to Control group (36.7%).

Discussion

In this study we decided to analyse both therapeutic effect of NAC on liver damaged by the long-term administration of CCl_4 as well as its possible additional harmful effects. According to the available information, there is needed further research on the impact of NAC supplementation on hepatic tissue under different conditions. This could confirm or refuse its application in clinical practice. Studies are necessary for the professionals to be sure about the effectiveness

Table 3. Total proteins, cholesterol and triacylglycerols in liver

	Control	Control+NAC	CCl_4	CCl_4+NAC
tProt (mg/g wt)	247.65 ± 11.75	222.39 ± 8.29	184.92 ± 12.27**	165.81 ± 8.73^{++}
tChol (mmol/kg)	4.34 ± 0.18	5.59 ± 0.37	10.81 ± 0.74**	10.27 ± 0.78^{++}
TAG (mmol/kg)	13.85 ± 1.47	11.10 ± 0.82	63.05 ± 10.56**	33.89 ± 4.28^{00+}

Data are presented as the mean ± SEM (n = 8); ** $p < 0.01$ *vs.* Control group; ^{00} $p < 0.01$ *vs.* CCl_4 group; + $p < 0.05$, ++ $p < 0.01$ *vs.* Control+NAC group. tProt, total protein; tChol, total cholesterol, TAG, triacylglycerols.

Table 4. Biochemical parameters of liver function in plasma

	Control ($n = 8$)	Control + NAC ($n = 8$)	CCl_4 ($n = 12$)	CCl_4 + NAC ($n = 10$)
ALT (μkat/l)	0.40 ± 0.03	0.39 ± 0.02	7.28 ± 1.18**	9.00 ± 2.05^{++}
AST (μkat/l)	1.19 ± 0.09	0.90 ± 0.07	7.19 ± 1.12**	10.07 ± 1.87^{++}
tBil (μmol/l)	0.83 ± 0.15	0.68 ± 0.09	8.87 ± 2.69**	2.56 ± 0.22^{oo}
tBile acids (μmol/l)	45.40 ± 12.62	21.25 ± 3.37	332.75 ± 63.67**	206.29 ± 31.03^{++o}
tProt (g/l)	63.52 ± 1.29	58.99 ± 1.66	55.86 ± 3.09*	55.02 ± 1.81
Alb (g/l)	27.90 ± 0.35	26.60 ± 0.84	23.48 ± 1.51**	23.99 ± 1.15
tChol (mmol/l)	1.37 ± 0.07	1.36 ± 0.08	0.80 ± 0.16**	1.34 ± 0.19^{o}
TAG (mmol/l)	1.30 ± 0.15	0.91 ± 0.05	0.61 ± 0.11**	0.83 ± 0.20
CHE (U/l)	50.75 ± 5.41	64.23 ± 7.26	75.14 ± 5.33*	98.06 ± 7.33^{o++}

Data are presented as the mean ± SEM; * $p < 0.05$, ** $p < 0.01$ *vs.* Control group; ^{o} $p < 0.05$, ^{oo} $p < 0.01$ *vs.* CCl_4 group; $^{++}$ $p < 0.01$ *vs.* Control+NAC group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; tBil, total bilirubin; tBile acids, total bile acids; tProt, total protein; Alb, albumin; tChol, total cholesterol; TAG, triacylglycerols; CHE, cholinesterase.

and safety of NAC prescription. In our experiment NAC had protective effects on some biochemical parameters. On the other hand, we observed its harmful effects. Concomitant administration of CCl_4 and NAC increased, even more than CCl_4 itself, cholinesterase activity. We also observed trend to increase AST and ALT activities in plasma and fibrosis in liver, what suggests the partially negative effects of NAC under these experimental conditions.

Effect of CCl_4

As expected, the long-term *intraperitoneal* administration of CCl_4 to experimental animals induced pathological changes in the liver. All examined parameters were significantly impaired. Reducing of body weight gain and increase of the ratio of liver weight to body weight (hepatosomatic index HI) confirmed the severe liver injury. Histopathological measurement confirmed the development of steatosis and fibrosis in animal liver after the 10-weeks CCl_4 administration. Increased activities of ALT and AST in plasma indicate harm of the hepatocytes plasma membrane primarily as a result of lipid peroxidation caused by $CCl_3^{\bullet}$ radical. This radical is formed during CCl_4 metabolism by the cytochrome oxidase system (Weber et al. 2003). The decreased concentrations of total proteins and albumin in plasma point to reduction of proteosynthetic capability of liver. We have observed elevated concentrations of TAG and cholesterol in the liver, while the levels of these lipids were reduced in plasma. According to Weber et al. (2003) review article, it is mainly caused by an impaired liver transport system, observed in CCl_4 model. Damaged transport of VLDL from hepatocytes confirms also results from hepatic cell cultures (adult rat hepatocytes by Kato and Nakazawa 1987). They explained it as a consequence of triacylglycerols accumulation following CCl_4 exposure and suppression of lysosomal acid lipase activity, which in addition takes place in the inhibition of VLDL-triacylglycerol secretion.

The fat accumulation in the liver may be associated with impaired synthesis of apolipoproteins which are required for the transport of VLDL from the liver into the blood stream. Elevation of AST activity indicates partially damaged mitochondria, observed also by other authors working with CCl_4 model (Krähenbühl et al. 1990), which may lead to the decrease of β-oxidation. This finding was confirmed in cell cultures also by Boll et al. (2001) and explained by the inactivation of cytochrome P-450 (*via* AMP-activated protein kinase signalling pathway). Reduced degradation of fatty acids, increased synthesis of TAG and disrupted transport observed in CCl_4 model could together lead to the accumulation of TAG in the liver.

Simultaneous increase of concentrations of both, the total bilirubin and total bile acids in the plasma, reflects the presence of cholestasis – impairment or complete dysfunction of hepatobiliar influx and efflux in the liver of rats in the CCl_4 experimental group. Under normal circumstances hepatocytes can take up bile acids from portal circulation leading to their low concentration in the systemic circulation. During necrotic changes of hepatocytes, it is possible to observe reduced uptake of bile acids by these cells. The increase in plasma concentration of bile acids observed in our study might be primarily explained by the attack of free radicals to cell membrane and secondarily also by retention of cholesterol resulting in increased membrane cholesterol content. The consequence of these events is the change in membrane fluidity and damage to transport mechanisms that ensure the uptake of bile acids from the blood. A similar mechanism is likely to cause an increase in the concentration of bilirubin, which is also taken up from circulation by the liver (specific carrier mechanism) to be subsequently conjugated in the hepatocytes and eliminated.

Cholinesterase activity reflects the liver synthetic capacity. However, its increased activity under the conditions associated with some degree of steatosis (obesity, hyperlipoproteinemia, non-alcoholic liver steatosis, diabetes) suggests another not known function and connection of this enzyme with other processes (Nomura et al. 1986; Thomas 2000; Turecký et al. 2005; Lunkes et al. 2006). Several authors observed a correlation between CHE activity and the TAG concentration (resp. VLDL and LDL); although finally no clear association was found (Annapurna et al. 1991; Abbott et al. 1993; Rustemujer 2001). In our work we have observed elevation of CHE activity in plasma but decrease in plasma TAG levels (Pearson correlation coefficient between CHE and TAG in plasma R = 0.291, NS) with a parallel rise in liver TAG levels (Pearson correlation coefficient between CHE and TAG in liver R = 0.441, $p = 0.05$).

Effect of NAC

We have found the improving effect of NAC on the plasma cholesterol level by decreasing its concentration to the level of the Control group. Similar positive effects of NAC on tBil and bile acids were found, however, the level of tBil was still higher by 208.43%, respectively by 354.38% for bile acids compared to the Control group. NAC might have had a protective effect on hepatocyte membrane and alleviated the damage to transport mechanisms responsible for the uptake of bilirubin and bile acids. The improvement of delivery systems by NAC could explain the normalization of plasma cholesterol and TAG in the liver.

There are studies which found the protective effects of NAC against CCl_4 toxicity in liver, however, with different design of the experiments. Cai et al. (2015) found decreased AST, ALT, necrosis and increased weight after NAC administration. Decreasing effect of NAC to TAG was observed by Maksimchik et al. (2008), however, ALT, AST and tBil were not affected. Our parameters indicating protective effects against CCl_4 (TAG in liver, total cholesterol and total bilirubin level in plasma) after NAC administration are consistent with the results reported by Kucharska et al. (2004) and Uličná et al. (2003). Both are made under similar conditions of the experiment. In addition, Kucharska et al. (2004) observed a significant positive effect of NAC also on liver cholesterol and Uličná et al. (2003) on histological parameters (steatosis, fibrosis) and ALT, AST activities in plasma. In their studies, unlike our, they administered NAC during 7 days before CCl_4 administration, which could make the liver to be more resistant to the injury. One of the possible explanations could be so called 'preconditioning' or 'hormesis'– a widely observed phenomenon in biomedical research (Mattson 2008; Son et al. 2008).

The different results on NAC effects depending on the start time of NAC administration (before resp. after induction of the damage) were also recorded by Xu et al. (2005) and Wang et al. (2006). Wang et al. found that NAC has a dual effect on acute ethanol-induced liver damage. The effects of NAC depend on the schedule of NAC administration. Pretreatment with NAC protects against acute ethanol-induced liver damage. On the other hand, NAC administered after damage induction, behaves as a pro-oxidant and worsens this kind of damage.

Even though NAC is known as an "antioxidant", it had been demonstrated that under certain conditions NAC and other thiol compounds can have pro-oxidant properties (Shen 2000; Sagrista 2002). According to Sprong et al. (1998) NAC behaves either as anti- or pro-oxidant dose-dependently. Low dose (275 mg/kg at 24 h before lipopolysacharides (LPS) challenge) of NAC protected rats against LPS-mediated oxidative stress, while high dose of NAC (900 mg/kg at 24 h before LPS challenge) increased LPS-induced lung injury and mortality.

Chan et al. (2001) found NAC to inhibit LPS-induced activation of the mitogen-activated protein kinases (MAPKs) in a serum-depleted environment (0.1% fetal bovine serum). By contrast, NAC enhanced LPS induction of p38 MAPK and JNK phosphorylation in the presence of 10% serum.

It is hypothesized that local redox environment might influence the effect of NAC. It could be supported also by results of Moura et al. (2016), who described negative effect of parallel NAC and lipoic acid administration to rats with induced mild ulcerative colitis. They observed increased level of H_2O_2 in the liver and ALT and AST activities in plasma were found, even as individually administered NAC or lipoic acid had protective effect.

"Auto-oxidizing" process of NAC is potentiated also by the presence of metals, such as Cu(II), and the presence of ROS, such as H_2O_2 (Oikawa et al. 1999). Furthermore, as thiol has undergone autoxidation, it no longer acts as an "antioxidant". Once initiated, these reactions can produce additional ROS including $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}OH$.

The molecular mechanisms by which NAC exerts its diverse effects are complex and still unclear (Samumi et al. 2013). As mentioned above, there are different theories partly explaining controversial results on NAC effects.

It should be more intensively discussed about change of generally accepted classification on "good" antioxidants to which NAC belongs and "bad" radicals (discussions published also on the website of the Nature journal). Another example pointing to the need for a change of view is also a similarly controversial situation in the issue of tumor cells. In tumor cells antioxidants (e.g. NAC) exhibited quite opposite effects. Li et al. (2016) reported that NAC has protected telomerase activity in normal cells but inhibited it in cancer cells. The authors suppose the opposite effects on telomerase activity results from different intracellular redox homeostasis in normal and tumor cells.

In conclusion, application of NAC in clinical practice has a lot of protective effects and they prevail, nevertheless its harmful effects were also found. Therefore, it is necessary to continue in monitoring of the NAC effects on various parameters characterizing the liver functions in dependence of dose, time and schedule of treatment. Studies are necessary for the professionals to be sure about the effectiveness and safety of NAC prescription.

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Conflict of interest. The authors declare that there are no conflicts of interest.

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