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Anti-inflammatory Effects of N-acetylcysteine against Carbosulfan-induced Hepatic Impairment in Male Rats

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Abstract
The present study is designed to investigate the anti-inflammatory potential of N-acetylcysteine (NAC) against carbosulfan (CB)-induced hepatic dysfunction in rats. Rats exposed to CB and NAC were examined for toxicity by assessing various biochemical alteration, inflammation (interferon gamma and leucocyte counts), and peroxidation marker, including in liver. Significant increases of blood alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyltransferase (GGT) were detected in CB-treated rats. In addition, the level of pro-oxidative marker, such as malondialdehyde (MDA), was also increased. According to the results, rats exposed to CB showed a significant alteration in hepatic enzymes and an increase in the accumulation of lipid peroxidation index; also, CB induced an increase in pro-inflammatory cytokine in CB-treated rats when compared with their respective controls. Interestingly, administration of NAC to CB-treated rats attenuates the toxicity of CB, objectified by biochemical and oxidative improvement of liver. Thus, the present study reports for the first time that NAC could be a promising therapeutic agent against CB-induced liver impairment.

Keywords: N-acetylcysteine; Carbosulfan; Liver; Inflammation.

1. INTRODUCTION

Anti-cholinesterase pesticides mainly include organophosphates, such as malathion, as well as carbamates, such as carbofuran and carbosulfan (CB). The acute toxicity of these pesticides has been well documented (Gupta, 1994; Lasram *et al*., 2009, 2014; Raheja and Gill, 2007; De Silva *et al*., 1992; Smulders *et al*., 2003). Liver, which is the main organ responsible for metabolism, is a common target of pesticide toxicity (Celik *et al*., 2009). Subchronic exposures to carbamate pesticides could induce hepatotoxicity with oxidative stress as the main mechanism (Binukumar *et al*., 2010; Kamboj *et al*., 2006).

It has been shown that oxidative stress, which is known to be involved in the pathogenesis of several diseases, has been described in acute and chronic exposure to carbamate pesticides (Gupta, 1994). The body has developed several defense mechanisms against oxidative damage. These defense mechanisms are composed of enzymatic and non-enzymatic systems (Eraslan *et al*., 2009). The enzymatic mechanism is made of free-radical scavengers, such as catalase, superoxide dismutase, and glutathione S-transferase (Durak *et al*., 2009; Khan and Kour, 2007). On the other hand, the non-enzymatic mechanism involves certain endogenous compounds found in the body and certain exogenous compounds taken into the body (vitamins E and C, flavonoids, etc.) (Eraslan *et al*., 2009; Uzun *et al*., 2010). It is reported that CB enhances the production of Reactive Oxygen Species (ROS), and it alters the enzyme activities associated with antioxidant defense mechanisms in the spleen of male Wistar rats (El-Bini *et al*., 2014).

Antioxidant drugs are becoming increasingly popular in oxidative stress-related disorders and hold promise as therapeutic agents (Borgstrom *et al*., 1986). N-acetylcysteine (NAC) acts as an antioxidant by restoring the pool of intracellular reduced glutathione, which is often depleted as a consequence of increased status of oxidative stress and inflammation (Grinberg *et al*., 2005). Furthermore, NAC also has reducing and antioxidant properties, acting as a direct scavenger of ROS (Sadowska *et al*., 2007). Therefore, considering that CB toxicity is associated with behavioral impairments and that NAC has important antioxidant actions, the aim of this study was to investigate the effects of this compound on liver function as well as oxidative stress and inflammation in tissue structures of CB-exposed rats.

2. MATERIALS AND METHODS

2.1. Animals

Rats (Wistar, 100-150 g of weight and 45 days of age) were procured from the Tunisian Society of Pharmaceutical Industries, divided into two groups, and housed two per cage in a sterile plastic cage. The vivarium was maintained under normal day/night schedule (12 h light/12 h dark cycles) at room temperature 25°C ± 1°C. Balanced food and

water were given to the animals *ad libitum*. All the procedures were in accordance with the Guidelines for Ethical Conduct in the Care and Use of Animals.

2.2. Chemical

CB was provided by the Tunisian Ministry of Agriculture; before use, CB was dissolved in corn oil for a final concentration of 25 mg/mL.

2.3. Treatment Schedule

A total of 36 animals were randomized into 3 groups of 12 rats each and were treated as follows for 30 consecutive days. CB or vehicle (corn oil) was administered in the morning (between 09:00 and 10:00 h) to non-fasted rats. The day of the first exposure to CB was delineated as experimental day 1.

Control group (CTR): One milliliter of corn oil at a dose per day was given via oral gavage to rats once a day.

CB-treated group: CB was given at the dose of 25 mg/kg of body weight (BW) dissolved in a total volume of 1 mL of corn oil once a day during 30 days.

CB + NAC (CB-NAC)-treated group: The rats received CB (25 mg/kg of BW) once a day and NAC (2 g/L) dissolved in drinking water during 30 days.

The choice of CB dose was based on previous works of our group (El-Bini *et al.*, 2014) and corresponded to an acceptable dose that did not cause any signs of toxicity until the end of the experiment period. The used dose of CB was calculated directly from commercial grade and corresponded to 1/10 LD50 (IPCS, 1996).

The dose of NAC was selected on the basis of previously published reports suggesting that NAC was not toxic to humans at a dose of 75 mg/kg or animals at this dose (Ortolani *et al.*, 2000). Furthermore, NAC does not show any signs of toxicity at doses even higher than the one administered in the present study (El Midaoui *et al.*, 2008).

2.4. Body Weight and Blood Collection

BW changes of male rats were recorded daily during the experimental period (30 days). At the end of this period, blood samples were withdrawn from the animals under ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary. Blood was collected into heparinized tubes and non-heparinized tubes and left for 20 min at room temperature. Then the tubes were centrifuged at 3,000 g for 10 min to separate the plasma or the serum. After that, the rats were sacrificed by cervical dislocation. Livers of male rats were quickly removed and weighted individually.

2.5. Clinical Hematological Leucocytes

Blood samples were collected into tubes with anticoagulant (EDTA) and immediately analyzed for hematological parameter (WBC) using an automatic hematological assay analyzer (BC-2800 VET Mindray Auto Hematology Analyzer, Mindray, China).

2.6. Plasma Biochemical Parameters

Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 3,000 g for 10 min and stored at –60°C. Stored plasma samples were analyzed for total protein (TP) by the biuret method according to Henry *et al.* (1978).

Glucose was measured by the glucose oxidase and peroxidase method using quinoneimine as a chromogen. The amount of plasma glucose is related to the amount of quinoneimine, which is measured spectrophotometrically at 505 nm (Lott and Turner, 1975).

The activities of cellular enzymes, such as aminotransferases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), lactate deshydrogenase (LDH), alkaline phosphatase (ALP), and acide phosphatase (ACP), were assessed with commercially available diagnostic kits supplied by Biomaghreb Laboratoires (Tunisia). Enzyme activity was expressed in international units per liter (IU/L).

Albumin (A) concentration was determined by the method of Westgard and Poquette (1972). Globulin (G) concentration was calculated as the difference between TP and albumin (Lowry *et al.*, 1951).

For determination of serum total cholesterol (TC) and triglyceride (TG) concentrations, the corresponding diagnostic kits, set by Biomaghreb Laboratories (Tunisia), were used according to the instructions of the manufacturer.

2.7. Hepatic Glycogen Dosage

Glycogen content was determined using the technique of Good *et al.* (1933). Briefly, 0.5 g of liver was extracted with 3 mL of 30% potassium hydroxide (KOH), incubated for 30 min at 100°C, and cooled and brought to acid pH by addition of 20% trichloroacetic acid (TCA). Precipitated protein was removed by centrifugation at 3,000 g for 10 min. Glycogen was precipitated by ethanol then weighed. The results are expressed in gram of glycogen per 100 g of liver.

2.8. Tissue Preparation

Livers were immediately removed, weighed, and washed using chilled saline solution. Tissues were minced and homogenized (10% w/v), separately, in ice-cold 1.15% in potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 g for 20 min at 4°C, and the resultant supernatant was used for enzyme assays. Protein concentrations in tissue were determined by the Coomassie reagent using serum bovine albumin as a standard (Bradford, 1976).

2.9. Oxidative Stress

Malondialdehyde (MDA), as a marker for lipid peroxidation (LPO), was determined in serum by the double-heating method of Begue and Aust (1978). The principle of the method is based on spectrophotometric measurement of the color produced during the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 mL of 100 g/L TCA solution was added into 0.5 mL tissue homogenate in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling under tap water, the mixture was centrifuged at 3,000 g for 10 min, and 2 mL of the supernatant was transferred into a test tube containing 1 mL of 6.7 g/L TBA solution and placed again in a boiling water bath for 15 min. The solution was then cooled under tap water and its absorbance was measured spectrophotometrically at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex (1.56×10^5 M/cm) and expressed in nmol/mL.

Total glutathione (GSH) content in tissue was measured by the method of Tietze (1969) using dithionitrobenzene and expressed as mmol/mL.

2.10. Cytokine Expression Analysis by RT-PCR

Total RNA was isolated from liver tissue using TRIzol reagent according to the manufacturer's instructions and then treated with DNase enzyme to remove any genomic DNA contamination. cDNA was synthesized from 1 µg of total RNA using oligo-dT and Superscript II Reverse Transcriptase (Invitrogen). cDNA (20 ng) was subjected to quantitative RT-PCR amplification using a LightCycler480 (Roche) using SYBR PrimeScript™ (Applied Biosystems). Only RNAs with RNA integrity number (RIN) N2 were used for reverse transcription and further processing. The forward and reverse primers for INF-γ rat gene is shown in Table 1. The thermal profile settings were 50°C for 2 min and 95°C for 2 min

Table 1: Effects of subchronic administration of CB/NAC on body weight, tissue weight (g), and tissue index of Wistar rats.

	CTR	CB	CB-NAC
Initial body weight (g)	152.41 ± 3.94	155.66 ± 5.84	158.33 ± 4.82
Final body weight (g)	255.25 ± 4.03	221.75 ± 4.58[a]	213.41 ± 3.84[a,b]
Mass gain (g)	62.83 ± 3.64	46.08 ± 5.99[a]	45.08 ± 2.69[a,b]
Food intake (g/kg b.w/day)	94.606 ± 3.422	75.375 ± 2.758[a]	66.483 ± 0.956[a]
Water intake (mL/kg b.w/day)	88.627 ± 2.059	81.07 ± 2.277[a]	78.62 ± 1.991[a]
Energy intake (Kcal/kg/j)	330.062 ± 11.93	262.971 ± 9.622[a]	221.947 ± 3.33[a]
Liver weight (g)	6.89 ± 0.41	9.06 ± 0.22[a]	6.152 ± 0.255[b]

CTR: Control group; CB: Carbosulfan-treated group; CB-NAC: Animals that received CB and NAC. Values are expressed as mean ± SD ($n = 12$).
a: significantly different from the control group ($p < 0.05$);
b: significantly different from the CB group ($p < 0.05$).

Table 2: Effects of subchronic administration of carbosulfan/NAC on blood glucose, cholesterol, and triglyceride level, and on hepatic glycogen rate.

	CTR	CB	CB-NAC
Glucose level (g/L)	1.01 ± 0.025	1.805 ± 0.167[a]	1.039 ± 0.057[b]
Hepatic glycogen rate (g of glycogen/100 g of liver)	7.008 ± 0.574	5.4 ± 0.277[a]	6.733 ± 0.738[b]
Cholesterol (mg/mL)	0.575 ± 0.029	0.671 ± 0.042[a]	0.647 ± 0.031
Triglyceride (mg/mL)	0.671 ± 0.039	0.823 ± 0.066[a]	0.832 ± 0.063

CTR: Control group; CB: Carbosulfan-treated group; CB-NAC: Animals that received CB and NAC. Values are expressed as mean ± SD ($n = 12$).
a: significantly different from the control group ($p < 0.05$);
b: significantly different from the CB group ($p < 0.05$).

and then 25-30 cycles at 95°C for 15 s and 60°C for 2 min. Relative expression levels of the mRNA of the target genes were normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA levels.

2.11. Statistical Analysis

Data were statistically analyzed using the Student's t-test to determine significant differences between different groups. Values of p less than 0.05 were considered significant. The values were expressed as means ± SD.

3. Results

3.1. Physical Observation

Obvious signs and symptoms of toxicity were not observed in the rats with any of the pesticide treatments. Also, none of the animals died.

3.2. Body Weight and Mass Gain

The changes in the body and organ weight of control and experimental rats during the experimental period are given in Table 1. There was no significant change in the initial BW between the groups, whereas there was a significant decrease in the final BW and mass gain and a significant increase in liver weight of CB-administered rats when compared with CTR rats. NAC-treated rats experienced increased final BW and mass gain and decreased liver weight after CB administration. However, it was important to indicate that death was not observed in any of the experimental groups during the experimental period. Also, no clinical signs of CB poisoning were observed among treated rats.

3.3. Changes in Plasma Glucose and Hepatic Glycogen Rate

CB at a dose of 25 mg/kg of body weight induced a significant increase in blood glucose (144.04%). The hepatic glycogen level was considerably decreased (22.94%). A significant decrease was observed in glycemia in the CB-NAC-treated group compared with CB-treated group by 42.43%. In addition, hepatic glycogen rate increased in the CB-NAC-treated group compared with CB-treated group by 135.09% (Table 2).

3.4. Changes in Lipid Status

In addition, CB increased significantly plasma cholesterol and TG content (Table 2). Interestingly, NAC affected significantly lipid status. Indeed, supplementation for 30 days of NAC normalized the level of TG while the levels changed

Table 3: Effects of subchronic administration of CB/NAC on the liver function markers of Wistar rats.

	CTR	CB	CB-NAC
AST (U/L)	85.7 ± 6.43	92.5 ± 5.44	89.239 ± 4.349
ALT (U/L)	40.9 ± 5.96	56.5 ± 5.73[a]	50.895 ± 2.94[a]
LDH (U/L)	1,014.3 ± 58.8	1,149.49 ± 117.2	1,144.47 ± 83.876
ALP (U/L)	222.41 ± 15.6	264.802 ± 19.864[a]	233.368 ± 29.216
ACP (U/L)	11.06 ± 0.57	7.001 ± 0.53[a]	8.658 ± 1.198[a,b]
GGT(U/L)	9.49 ± 1.34	17.037 ± 1.742[a]	12.58 ± 1.079[b]

CTR: Control group; CB: Carbosulfan-treated group; CB-NAC: Animals that received CB and NAC; AST: ; ALT: Alanine aminotransferase; LDH: Lactate deshydrogenase; ALP: Alkaline phosphatase; ACP: Acide phosphatase; GGT: Gamma glutamyltransferase. Values are expressed as mean ± SD ($n = 12$).
a: significantly different from the control group ($p < 0.05$);
b: significantly different from the CB group ($p < 0.05$).

Table 4: Effects of subchronic administration of CB/NAC on hepatic protein of Wistar rats.

	CTR	CB	CB-NAC
Serum Total Protein (g/dl)	71.886 ± 0.945	87.318 ± 0.62[a]	70.861 ± 1.09[b]
Albumine (g/dl)	47.108 ± 1.247	30.915 ± 1.954[a]	47.888 ± 0.511[b]
Globuline (g/dl)	21.977 ± 1.501	41.21 ± 1.33[a]	25.258 ± 0.847[b]

CTR: Control group; CB: Carbosulfan-treated group; CB-NAC: Animals that received CB and NAC. Values are expressed as mean ± SD ($n = 12$).
a: significantly different from the control group ($p < 0.05$);
b: significantly different from the CB group ($p < 0.05$).

in the CB-NAC-treated group compared with CB-treated group as shown in Figure 2. On the contrary, TC was not affected by NAC addition (Table 2).

3.5. Liver Dysfunction

The data demonstrating the level of liver damage sustained following exposure to CB are shown in Tables 3 and 4. In order to determine whether the CB dosing (25 mg/kg) produced toxicity to animals, we measured plasma AST, ALT, LDH, ALP, and gamma glutamyltransferase (GGT) activities. Table 3 shows that CB increased the activity of those enzymes ALT, ALP, and GGT in plasma. This evidence suggests that the given CB dose is able to induce some degree of liver toxicity. Also, CB caused a significant decrease in the albumin level. Long-term NAC treatment, which did not affect plasma ALT and ALP activity, was able to reverse CB-induced increase in plasma GGT activity. As shown in Table 4, significant increases in TP and globulin concentrations were observed in the serum of CB-treated rats, and a significant decrease in the albumin level was also noted, in comparison with the results of control animals. Moreover, CB-NAC treatment reversed TP and globulin levels to within the normal limits. On the other hand, NAC produced a slight reduction in CB-induced albumine release, but it was marginally significant ($p = 0.075$).

3.6. Oxidative Stress

The liver LPO level of CB-treated rats varied significantly during the 30 days of experiment (Figure 1). The LPO level was higher in the liver of CB-treated group. The supplementation of NAC in CB-treated rats brought down the hepatic LPO to the level comparable with those of CTR rats. Conversely, as depicted in Figure 2, the levels of glutathione (GSH) in rats treated with CB for 30 days significantly decreased in liver by 26.55% in comparison with the CTR. A significant CB-NAC interaction for tissue GSH levels was observed. Results demonstrate an increase in GSH by 125% in liver (Figure 2).

Figure 1: Effects of subchronic administration of carbosulfan (CB)/N-acetylcysteine (NAC) on liver malondialdehyde (MDA) level. Values are expressed as mean ± SD ($n = 12$). a: significantly different from the control group ($p < 0.05$); b: significantly different from the CB group ($p < 0.05$).

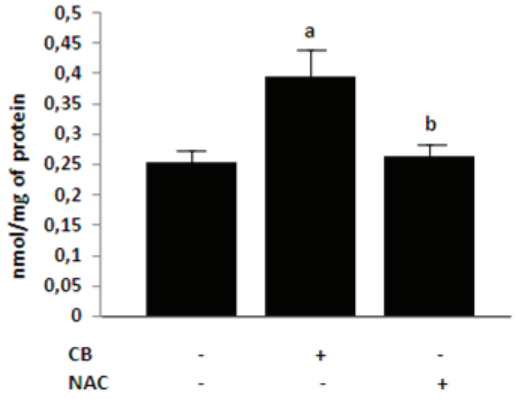


Figure 2: Effects of subchronic administration of carbosulfan (CB)/N-acetylcysteine (NAC) on liver glutathione (GSH) level. Values are expressed as mean ± SD ($n = 12$). a: significantly different from the control group ($p < 0.05$); b: significantly different from the CB group ($p < 0.05$).

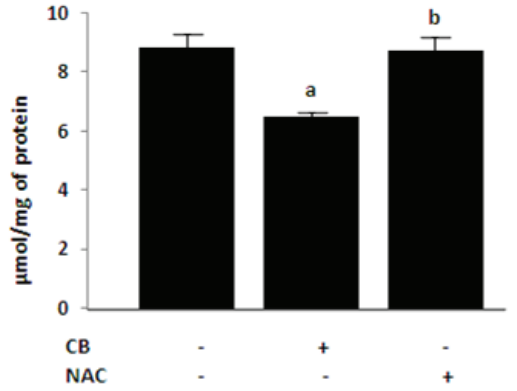


Figure 3: Effects of subchronic administration of carbosulfan (CB)/N-acetylcysteine (NAC) on blood leucocyte level. Values are expressed as mean ± SD (n = 12). a: significantly different from the control group ($p < 0.05$); b: significantly different from the CB group ($p < 0.05$).

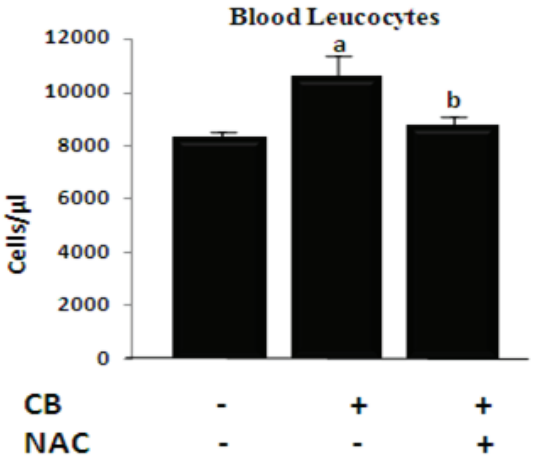
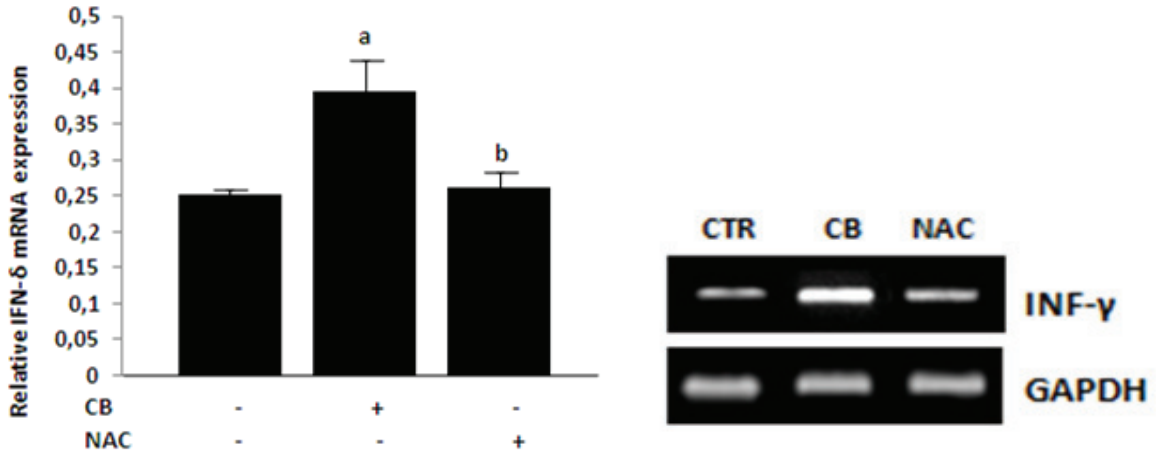


Figure 4: Effects of subchronic administration of carbosulfan (CB)/N-acetylcysteine (NAC) on liver INF-δ mRNA expression of experimental rats. Values are expressed as mean ± SD (n = 12). a: significantly different from the control group ($p < 0.05$); b: significantly different from the CB group ($p < 0.05$).



3.7. Change in Leukocyte Level

CB significantly increased WBC counts (28.42%) compared with the CTR (Figure 3). Interestingly, NAC administration resulted in a significant reduction in WBC counts in the CTR (17.51%) as compared with the CB-treated group.

3.8. Cytokine Expression

The mRNA expression of hepatic pro-inflammatory cytokines is shown in Figure 4. Quantitative mRNA analyses have demonstrated an up-regulation of pro-inflammatory cytokine mRNA IFN-γ expression in the liver of CB-treated animals. NAC supplementation during the CB treatment significantly decreased the mRNA levels of liver pro-inflammatory cytokine compared with the treatment with CB alone.

4. DISCUSSION

It has been previously reported that NAC is able to protect against CB-induced increases in oxidative markers of rat spleen (El-Bini *et al.*, 2014). In line with these reports, the present study demonstrates the beneficial effect of a subchronic NAC treatment (2 g/L in drinking water) against CB-induced impairment in inflammatory markers in the rat liver. In a previous study, we showed that *in vivo* subchronic treatment with NAC protects the spleen against CB toxicity

and recovers from oxidative stress induced by CB (El-Bini *et al*., 2014). In the present study, we demonstrated that oral NAC administration leads to a reversion of carbamate effects by preventing CB-induced increases in pro-inflammatory markers and hepatic damage. Since carbamate intoxication represents an important human health problem (Flessel *et al*., 1993; Gupta, 1994) and one of the major targets for carbamate toxicity in the liver that besides metabolic disorders. Our study constitutes an important contribution regarding a preventive treatment against the CB-induced impairment in hepatic dysfunction.

In toxicological studies, body and organ weights are important criteria for the evaluation of toxicity. In the present study, the final BW of CB-treated rats was significantly ($p < 0.05$) lower than that of the CTR. This may be attributed to a decreased food intake (anorexia or food avoidance), poor food palatability, or increased degradation of lipids and protein due to treatment-related toxicity (Mansour and Mossa, 2010). Also, weight loss observed in the CB-treated group may be a result of the combination of oxidative stress and adrenal-mediated stress caused by the inhibition of cholesterol ester hydrolase. CB is known to show its toxic effects by inhibiting cholinesterase activity, which could be due to the less food consumption and/or fluid and electrolyte loss (El-Bini *et al*., 2014; Yi *et al*., 2006). These data are in consonance with those obtained with organophosphorous pesticides by Ambali *et al*. (2007) and Al-Othman *et al*. (2012). Results showed that there is a significant increase in the relative liver weight in rats exposed to CB compared with control rats (Table 1). The increase in relative organ weight in rats exposed to carbamate pesticides seems to be due to their toxic potential and a marker of inflammation, and it is in agreement with the results obtained by Al-Sarat *et al*. (2011) in rabbits and Institoris *et al*. (2002) in rats.

Curiously, the energy intake was significantly similar in the two groups, CB and CB-NAC (Table 1), while enhanced food and water intake were only observed in CB rats. This observation suggested that dietary factors other than energy intake play important role in BW regulation. Note that the final BW and the mass gain was lowest in CB rats and that NAC normalized those parameters in the CB-NAC group (Table 1), indicating that one mechanism by which NAC ameliorates the BW gain was by modified energy expenditure. On the other hand, NAC attenuated the increases in liver weight caused by CB. NAC is known for its chelation potential, and supplementation of NAC during pesticide intoxication reduced the levels of CB in the liver. In consistent with our results, studies of De Flora *et al*. (1986) reported that NAC lowered the levels of ethyl carbamate in tissues of mice.

In the present study, we assess the hepatic function by determination of blood glucose level and lipid markers, because the regulation of glucose and lipid metabolism is mainly in the liver. Indeed, CB treatment produced significant increase in plasma glucose levels. This rise in glycemia was along with reduction in BW, as shown in Table 2. NAC treatment significantly reduced the plasma glucose levels as compared with the CB-treated group. The partial lowering of glucose levels by NAC might be attributable to its effect in preventing hyperglycemia-induced insulin resistance (Haber *et al*., 2003), since NAC has not been seen to increase plasma insulin levels in the diabetic rats (Franzini *et al*., 2008; Xia *et al*., 2007). According to our results, CB stimulates glycogenolysis pathway. Thus, treated rats show a lower level of liver glycogen. Similar results have been reported in rats after oral administration of diazinon (Teimouri *et al*., 2006) and acephate (Joshi and Rajini, 2009). Hepatic glucose utilization requires glucokinase activity, which plays a crucial role in the homeostasis of glucose and glycogen metabolism (Pilkis and Granner, 1992). Additionally, NAC supplementation affects hepatic glycogen level after CB exposure. To the best of our knowledge, this is the first study showing the effects of chronic antioxidant therapy, with NAC's impact on the hepatic glycogen level changes induced by carbamate pesticides.

On the other hand, results from the serum lipid status of rats treated with CB showed significantly increased concentrations of serum cholesterol (TC) and TG levels. These alterations were associated with the increase of cardiovascular risk factors and the atherogenic index. Accordingly, Kalender *et al*. (2010) also reported that carbamate pesticides increased plasma TG levels, which were attributed to an inhibition of the lipase activity of both the hepatic TGs and plasma lipoproteins. Underlying mechanisms of CB-induced hyperlipidemia are yet to be elucidated, and at the moment, it is difficult to establish whether there is a relationship between hyperlipidemic and hyperglycemic effects of CB. However, one can speculate that if these alterations remain over time, the CB exposure may represent a risk factor for the development of cardiovascular diseases. NAC effectively reduced the elevated lipid levels increased by CB exposure and restored their activities. These results suggest that NAC is able to fully protect liver from injury produced by CB.

According to our results, the CB dose used in the present study caused a rise in plasma ALP and GGT activity. In parallel, it was possible to observe an increase in ALT activity, which was a marker of hepatic toxicity. Altogether, these data point to a significant level of systemic toxicity for the CB dosage used. Regarding ACP and ALP activities in rat blood, it is clear that treatment with CB significantly decreased ACP activity but increased ALP as compared with controls. Also, Igwenyi *et al*. (2014) reported a significant increase in ALP activity, in the serum and liver of experimental animals treated with Baygon (pesticide brand). ACP is known to be localized in lysosomes and surrounded by a lipoprotein membrane. The decrease in ACP may be related either to the leakage of the enzyme into extracellular compartments or to tissue damage (Ambali *et al*., 2007). ACP was used as a marker for lysosomal functions in liver cells in order to estimate the interference with catabolic and autophagic processes in the liver. Also, the change in the ACP activity may be related to the biotransformation and elimination of the tested pesticide (Khan, 2014). The significant elevation in the activity of

ALP indicated damage to any or all of the organs producing this enzyme, such as the liver. This is due to the leakage of lysostic enzymes into the cytoplasm and renal necrosis and pyknotic nuclei. This result is paralleled by the result of Ambali *et al*. (2007), and Rahman *et al*. (2002) suggested that the alteration in the activities of ALP and ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, showing the stress condition of the treated animals. In addition, the alteration in these enzymes may be due to liver dysfunction, and disturbance in the biosynthesis of these enzymes with change in the permeability of liver membrane takes place. Moreover, CB-induced hepatic impairment is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. On the other hand, NAC treatment was unable to completely reverse CB-induced ALT and ALP increase; however, it caused some degree of hepatic protection by recovering plasma ACP and GGT activity, as shown in Table 2.

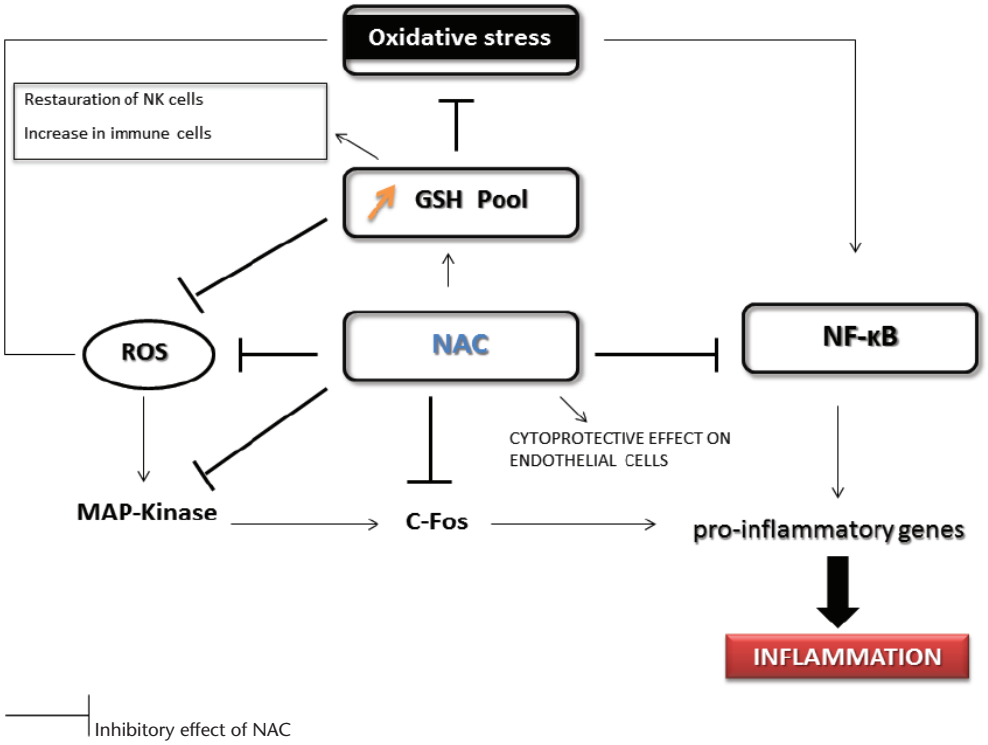
In this study, the level of TP and globulin was significantly increased in CB-treated animals. Moreover, the albumin level decreased in the CB-treated group. Also, several studies have shown that albumin production by liver can be decreased under pesticide exposure (Kalender *et al*., 2010; Yousef *et al*., 2003). The albumin level may decrease in individuals that liver function disorders after CB treatment. Albumin most often transports or binds drugs or chemicals (Kalender *et al*., 2010). Some insecticides increase the TC and TG levels (Kalender *et al*., 2010). The changes of TP level is based on the function of albumin and globulin proportion, which could vary based on immunocompetence "status" of the animals or other physio-pathological condition (Petterino and Argentino-Storino, 2006). Thus, increased serum cholesterol and TG levels can be attributed to the effects of the pesticide on the permeability of liver cell membranes (Yousef *et al*., 2003). Also, increase in serum cholesterol and GGT after subchronic CB exposure directly indicates the hepatic injury. The formation of fatty lipid steatosis after CB administration is mainly due to accumulation of TGs. The reason for the altered activities of marker enzymes during the toxicity might be due to the fact that oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species (O_2, H_2O_2, and OH) exceeds beyond the antioxidant capability of the cell (Demiryilmaz *et al*., 2012). Ksheerasagar and Kaliwal (2010) have also observed similar type of results in rat serum when mice livers were treated with CB. A significant reduction in liver function markers toward respective control values by NAC is an indication of the stabilization of the plasma membranes of the hepatic tissue caused by CB.

Functional alteration mechanism in the liver can be due to the presence of an inflammatory condition or oxidative stress. In fact, in the present study, CB-treated rats also exhibited significantly increased WBC counts. This increase in WBC may indicate an activation of the animal's defense mechanisms and immune system. It could be related to the liver damage and result from an inflammation caused by CB liver toxicity (Kandil *et al*., 2006). These changes and mechanisms also related to increase of ALP and ALT activities in serum because their levels in serum show that cellular degeneration or destruction occurs in liver (Elhalwagy *et al*., 2008). Interestingly, NAC treatment caused a significant decrease in total WBC values. Our results corroborate with those of De la Fuente and Hernanz (2012), who showed that NAC *in vitro* improves the number and the functions of leucocytes in old mice.

Also, initial injury from the toxic metabolites of CB activates the Kupffer cells in liver, leading to the release of pro-inflammatory cytokines, such as INF-γ. The role of inflammatory responses in toxicological process is of considerable interest since these are involved in liver injury by promoting hepatosteatosis (Andrés *et al*., 2003). Injury to hepatocytes causes the activation of adjacent non-parenchymal cells (Kupffer cells and stellate cells) resulting in the production of cytokines. IFN-γ induction occurs within minutes following exposure (Luster *et al*., 2000). These cytokines are responsible for the activation of nuclear transcription factors, such as nuclear factor κB, and are involved in hepatic inflammation and apoptosis of damaged cells (Jaruga *et al*., 2004). On the other hand, increase of leukocyte levels is the certain consequence of enhancement of cytokine expression that likely contributed to the increased levels of IFN-δ in the liver of CB-treated rats. Our results are in agreement with experimental studies, which showed that organophosphate compounds can directly increase expression of pro-inflammatory cytokines. Indeed, the increase in pro-inflammatory cytokines, such as TNF-α and IL-10, was observed in rats treated with diazinon (Hariri *et al*., 2010) and endosulfan (Ayud *et al*., 2003). Cytokines possess a diverse array of functions, including the ability to activate macrophages, promote leukocyte recruitment, stimulate B-cell and T-cell differentiation, and increase vascular permeability (Banks and Lein, 2012). In our study, NAC reduced the levels of the pro-inflammatory cytokine IFN-δ. Furthermore, NAC decreased the levels of pro-inflammatory cytokines IL-6 and IL-10 in Lipopolysaccharide (LPS)-treated rat fetal brain (Beloosesky *et al*., 2012). This raises the possibility that NAC promotes the synthesis of certain proteins that inhibit the activation of IKκβ. The effects of NAC may be dependent on the synthesis of glutathione, suggesting that inhibition of NF-κB by the suppression of IKκβ could be the result of the indirect action of NAC. However, this does not necessarily exclude the role of free radicals in the modulation of NF-κB activity.

Another mechanism of hepatic injury during CB exposure is the development of oxidative stress due to generation of reactive oxygen species. In the current study, CB toxicity was evidenced by determination of liver function tests, markers of oxidative stress by estimation of LPO level and GSH content. Cells under oxidative stress display various dysfunctions due to damage caused by reactive oxygen species to lipids, proteins and DNA. Also, oxidative stress in cells can be partially responsible for the toxic effects of carbamate pesticides. CB induces spleen toxicity in part by increasing of free-radical production (El-Bini *et al*., 2014). LPO is known to be one of the molecular mechanisms for cell injury in pesticide poisoning (Kaur and Sandhir, 2006; Lasram *et al*., 2014a). Enhanced levels of hydroxyl radical

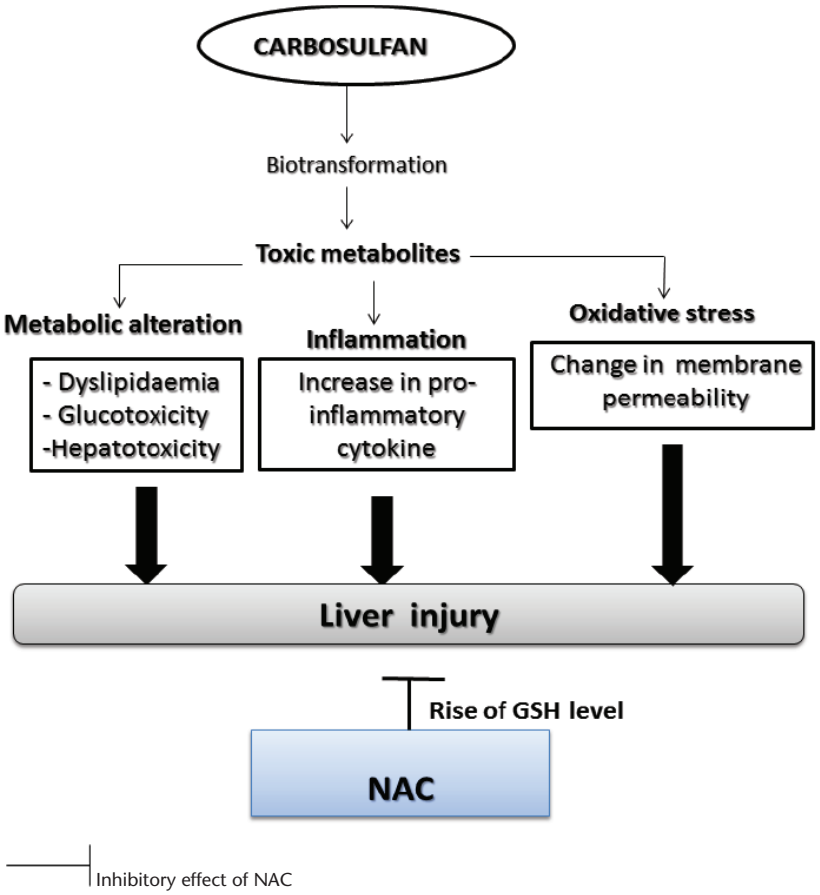
Figure 5: Anti-inflammatory effect of N-acetylcysteine (NAC) against cell damage induced by carbosulfan in rat liver cells. Carbosulfan acts directly on hepatocytes to induce liver injury and inflammation. Tissue damages sensitize hepatic cells to release enzymes in the blood. NAC treatment also augments intracellular glutathione (GSH) concentration, which modulates liver function.



also initiate MDA levels to rise in the liver regions. Similar reports have shown an elevation in the status of LPO in liver during carbofuran (Kaur and Sandhir, 2006) and carbaryl exposure (El-Demerdash *et al*., 2013), and our results are in accordance with these reports.

In the literature, CB is shown as a direct cholinesterase inhibitor and is converted to its toxic form by cytochrome P450-mediated reactions (Abbas *et al*., 2009). In the present study, we have observed that the depletion of the levels of non-enzymic antioxidants, such as GSH, due to excessive organ damage and oxidative stress is caused by CB toxicity. GSH is the major thiol, which binds electrophilic molecular species and free-radical intermediates. It plays a central role in the antioxidant defense system, metabolism, and detoxification of exogenous and endogenous substances (Eraslan *et al*., 2009). Also, GSH with its SH group functions as a catalyst for disulfide exchange reactions, and contributes in H_2O_2 detoxification. Thiols, together with non-enzymatic and enzymatic factors, regulate the intracellular metabolism defending biological structures and functions from the noxious attack by ROS (Di Simplicio *et al*., 1998). In fact, in oxidative conditions, GSH is readily oxidized to Glutathione disulfide (GSSG), which may react with the SH group of proteins to form mixed disulfide through thiol/disulfide exchange. Carbamate pesticide has high affinity for endogenous thiol molecules, such as GSH, and CB toxicity causes irreversible binding with up to two GSH tripeptides (El-Demerdash *et al*., 2013). The conjugation of CB with GSH molecule process is desirable in that it results in the excretion of toxic metabolites, such as carbofuran, into bile. In the present investigation, it was observed that CB intoxication significantly depletes the GSH content in liver and thus reducing the antioxidant potential and accelerating the LPO, resulting in hepatic damage. Treatments with NAC attenuated the CB-induced oxidative damage. In fact, NAC restored the increased MDA and reduced GSH levels to the normal values in hepatic tissue. This could be attributed to the excellent properties of NAC. This property seems to be due to its ability to scavenge free radicals. It is reported that antioxidants may enhance the property of chelating agent, as evidenced by our group, against other forms of CB toxicity that are as dangerous as pesticide toxicity. NAC acts directly as a free-radical scavenger and precursor of reduced glutathione and glutathione peroxidase enzyme (Cotgreave, 1997). The present study demonstrates the therapeutic role of NAC, and it may be an ideal therapeutic agent in therapy for use in subchronic CB toxicity. NAC has antioxidant and anti-inflammatory property (Figure 5), and it reacts most strongly with hydroxyl radical and with hypochloric acid, but reacts poorly with hydrogen peroxide and superoxide radicals (Arakawa and Ito, 2007). It may also exert an indirect antioxidant effect by facilitating GSH biosynthesis and supplying GSH for

Figure 6: Preventive effects of N-acetylcysteine against cell damage induced by carbosulfan in rat liver cells. Supplementation of N-acetylcysteine treatment modulates liver function.



GSH-Px-catalyzed reactions (Saricaoglu *et al.*, 2005). GSH-Px converts hydrogen peroxide to water (Halliwell 2006). However, liver GSH levels in the current study were increased by NAC administration because of facilitating GSH biosynthesis. A number of researchers suggested that NAC is effective as a chelating agent in reducing the toxicity of pesticide when administrated in intoxicated animals (Borgstrom *et al.*, 1986; Lasram *et al.*, 2014b; Sevgiler *et al.*, 2007; Zimet, 1988).

5. CONCLUSIONS

In conclusion, our results indicate that NAC is able to recover CB-induced impairment, showing to be a potential agent to be used against the pro-oxidative effects of carbamate compounds, such as CB (Figure 6). In addition, the data show that NAC attenuates the pathogenesis of the liver-cell injury induced by CB. There is evidence that the stabilization of free radicals play a crucial role in the protective mechanisms of NAC against CB toxicity (Figure 6). The interactions between CB and NAC are still obscure; also, evidences of molecular mechanism need more *in vivo* and *in vitro* investigations since evidences are not available in the literature.

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