

E-ISSN: 2378-654X

Recent Advances in Biology
and Medicine

Original Research Article

Protective Effect of
N-Acetylcysteine Against
Toxicity on the Rat Blood
After Chronic Exposure
to Carbosulfan

HATASO, USA

Protective Effect of N-Acetylcysteine Against Toxicity on the Rat Blood After Chronic Exposure to Carbosulfan

Ines El-Bini Dhouib*, Alya Annabi, Aicha Jrad, Najoua Gharbi,
Mohamed Montassar Lasram, Saloua El-Fazaa

Department of Biology, Faculty of Sciences, Laboratory of Aggression Physiology
and Endocrine Metabolic Studies, Tunis, Tunisia.

*Correspondence: inesbini@yahoo.fr

Received: Jan 30, 2015; Accepted: Mar 5, 2015; Published: Mar 13, 2015

Abstract

The present study investigated the protective effects of N-acetylcysteine (NAC), is widely known as an antidote to acetaminophen overdose, on carbosulfan (CB)-induced hematotoxicity and oxidative stress in male rats. CB was administered at a dose of 25 mg/kg or simultaneously administered with NAC (2 g/l) for 30 days. Results of hematological examination showed that red blood cells, hematocrite, hemoglobin, and reticulocytes levels were significantly lower in CB-exposed rats compared with those in the control. Administration of CB caused a significant increase in the superoxide dismutase and catalase activities. However, the glutathione (GSH) and thiols group (TSH) levels were significantly increased as well as GSH S-transferase activity and levels of glutathione peroxidase on erythrocytes of males rats compared with those in the control. Also, CB-treated rats showed significant elevation in lipid peroxidation (LPO) and acetylcholinesterase (AChE) on erythrocytes in comparison with the control. Co-administration with NAC exhibited chemoprotective effects against CB-mediated hematotoxicity, augmented erythrocyte antioxidant status, and prevented the induction of anemia.

Keywords: N-acetylcysteine; Carbosulfan; Rat erythrocyte; Oxidative stress.

1. INTRODUCTION

Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution and, therefore, are a cause of concern. Residual amounts of carbamates pesticides have been detected in the soil, water bodies, vegetables, grains, and other foods products (Abdollahi *et al.*, 2004). Carbamates are known to cause inhibition of acetylcholinesterase (AChE) activity in the target tissues (Abdollahi *et al.*, 1996) which accumulates acetylcholine and prevents the smooth transmission of nerve functions leading to convulsions and death. However, low intakes of carbamate through food and water may not show clear symptoms of OP intoxication such as convulsions but it may show mild inhibition of AChE activity in erythrocytes and tissues. Recent studies indicate that carbosulfan (CB) intoxication produce oxidative stress by the generation of free radicals and induce lipid peroxidation (LPO) in the rat spleen (El-Bini Dhouib *et al.*, 2014) and in the fish liver (Capkin *et al.*, 2014). As some of the carbamates may be present in blood of exposed humans and animals, it may produce oxidative stress in erythrocytes. The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) as well as total-SH content in erythrocytes, however, may neutralize the oxidative stress. N-acetylcysteine (NAC) is a cysteine prodrug and can be considered a GSH precursor (Rushworth and Megson, 2014) and oral administration of NAC replenishes the cellular levels of GSH (Atkuri *et al.*, 2007). High-dose oral of NAC is an effective antioxidant and as it is present at the site of free radical generation, it may neutralize the toxic effects of reactive oxygen species (ROS) (Aruoma *et al.*, 1989).

It has not been studied whether NAC modify the alterations induced by CB in antioxidant enzyme system and LPO concentrations in the rat erythrocyte. Hence, we aimed to evaluate whether there would be protective effect of NAC on hematological parameters, oxidative stress, and antioxidants values in CB-induced hematotoxicity in rats.

2. MATERIALS AND METHODS

2.1. Animals

Rats (Wistar, 100-150 g of weight and 45 days of age) procured from Tunisian Society of Pharmaceutical Industries, divided into two groups, and housed two per cage in sterile plastic cage. The vivarium was maintained under normal day/night schedule (12 h light/12 h dark cycles) at room temperature $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Balanced food and water were given to the animals *ad libitum*. All the procedures were in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

2.2. Chemical

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

2.3. Treatment Schedule

The rats were randomly divided into three groups of 12 animals each. The first group (control group: CTR) received orally 1 ml corn oil via stomach tube daily during 30 days. The second group (CB) received an amount of 1 ml corn oil containing 25 mg of CB/kg body weight/day. The last group (NAC) received 25 mg of CB/kg body weight/day and 2 g/l of N-acetylcysteine ($C_5H_9NO_3S$) in drinking water. The choice of CB dose was based on previous experimental assays in our laboratory, which corresponds to an acceptable dose that did not cause any sign of toxicity until the end of the experiments. This dose corresponds to 20% of LD50 for rat (Umetsu and Fukuto, 1982).

The dose of NAC was selected on the basis of previously published reports suggesting that NAC was not toxic to humans 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. Blood Collection

At day 30 and 2 h after the last dose, blood was collected from the retro-orbital plexus of each rat using heparinized capillary tubes for determination of the Acetylcholinesterase (AChE) activity and EDTA (Ethylene Tetra Acetic Acid) tubes for hematological parameters determination. The blood was centrifuged and the erythrocytes were washed twice with 0.1 M phosphate buffered saline (PBS, 1:9), pH 7.4. Erythrocyte lysate was prepared according to the method of McCord and Fridovich (1969) for the assay of oxidative stress parameters (Mates *et al.*, 1999b).

2.5. Acetylcholinesterase Activity in Erythrocytes

AChE activity was assayed by the method of Ellman *et al.* (1961). Hemoglobin (Hb) was estimated using Drabkin's reagent by the method of Dacie and Lewis (1968).

2.6. Determination of Blood Cellularity

Blood samples were analyzed immediately for blood cellularity using an automatic hematological assay analyzer (BC-2800 VET Mindray Auto Hematology Analyzer, Mindray, China). Routine hematological parameters were assessed: RBC (Red Blood Cell), Hb (Hemoglobin), Ht (Hematocrite), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), and Reticulocytes.

2.7. Lipid peroxidation

LPO is detected by the determination of malondialdehyde (MDA) production determined by the method of Begue and Aust (1978). Erythrocytes were centrifuged at $10,000 \times g$ for 10 min at $4^\circ C$ to sediment cell debris and mitochondrial samples, were suspended in PBS (0.1 M; pH 7.4), mixed with BHT-TCA (butylhydroxytoluene, trichloroacetic acid) solution (1% (w/v) BHT dissolved in 20% TCA) and centrifuged at $3000 \times g$ for 35 min. Then, the supernatant was mixed with 0.5 NaCl and 120 mM TBA (thiobarbituric acid) in 26 mM Tris and heated in water bath at $80^\circ C$ for 10 min. After cooling, we determined the absorbance of the resulting chromophore at 532 nm. MDA levels were determined by using an extinction coefficient for MDA-TBA complex of $1.56 \times 10^5 M^{-1} cm^{-1}$.

2.8. Determination of Thiol Groups Concentration

Erythrocytes are added to 0.25 M base Tris and 20 mM EDTA pH 8.2 (Hu and Dillard, 1994). Then, the mixture was vortexed and its absorbance was determined at 412 nm. The first value was noted A1. After that, 10 mM DTNB (5,5-dithiobis, 2-nitrobenzoate) were added and incubated for an incubation period of 15 min and a new value A2 was determined. The white tube of DTNB contains only DTNB and buffer; its absorbance value is noted as B. We calculated the concentration of thiol groups (TSH) per tube by using this equation expression: $(A2-A1-B) \times 1.57$ mM.

2.9. Antioxidant Enzymes Activities

The activity of SOD in erythrocytes of control and treated rats was assayed by the spectrophotometric method. CAT activity was measured at $20^\circ C$ by a slightly modified method of Aebi (1984). Hydrogen peroxide (H_2O_2) decomposition by CAT enzyme was monitored kinetically at 240 nm. One unit of activity is equal to the micromole of H_2O_2 degraded per minute per milligram of Hb.

2.10. Glutathione and Related Enzymes

Total glutathione (GSH) content in tissue was measured by the method of Tietze (1969) using dithionitrobenzene and expressed as mmol/ml. Glutathione peroxidase (GPx) activity was measured by the Wendel method, using tertbutylhydroperoxide as a substrate (Wendel, 1981). Nicotinamide adenine dinucleotide phosphate (NADPH) disappearance was monitored by a spectrophotometer at 340 nm. Glutathione S-transferase (GST) activity was assayed by the procedure of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The assay was conducted by monitoring the appearance of the conjugated complex of CDNB and GSH at 340 nm.

2.11. Statistical Analysis

Data were statistically analyzed using the Student “t” test to determine significant differences between different groups. *p* values less than 0.05 were considered significant. The values are expressed as means \pm SD.

3. RESULTS

3.1. Influence of NAC on the CB-Induced Hematotoxicity

The mean values of hematological parameters in rats exposed to CB alone and CB plus NAC are presented in Table 1. Rats administered with CB had significant alteration in RBCs, Hb, hematocrit, and reticulocytes values when compared with those in the control group. Co-administration with NAC significantly increased the values of RBC, Hb, and reticulocytes values to near CB group.

3.2. Erythrocyte AChE Activity

AChE activity in erythrocyte is shown in Figure 1. There was a significant decrease in AChE activity by 43.78% after 30 days treatment ($p < 0.01$) in CB-treated group. On the other hand, NAC treatment resulted in partial recovery of AChE activity (127.7%) in erythrocyte as compared to the CB treated animals.

3.3. Erythrocyte Oxidative Stress

3.3.1. LPO

Effect of *in vivo* the co-administration of CB and NAC on the LPO levels in the erythrocytes are presented in Figure 2. CB treatment resulted in a significant decrease in MDA level (30.9%) as compared to the control animals. On the other hand, NAC treatment resulted in a partial recovery of MDA level (127.5%) as compared to the CB-treated animals.

Table 1: Effect of NAC on CB-induced hematotoxicity in rats following 30 days exposure.

	CTR	CB	NAC
RBC ($10^{12}/l$)	6.7 \pm 1.01	8.278 \pm 0.829	8.036 \pm 0.551
Hb (g/l)	131.8 \pm 12.39	160.333 \pm 3.617 ^a	144.5 \pm 5.779 ^b
Ht (%)	39.78 \pm 4.38	48.1 \pm 1.677 ^a	46.35 \pm 2.371
MCV (fL)	61.3 \pm 4.753	60.06 \pm 4.853	60.1 \pm 2.971
MCH (pg)	18.4 \pm 1.37	17.6 \pm 0.41	18.35 \pm 1.675
MCHC (g/l)	323.25 \pm 10	313.7 \pm 2.495	317.666 \pm 3.747
Reticulocytes (%)	2.98 \pm 0.23	1.36 \pm 0.15 ^a	2.5 \pm 0.22 ^b

CTR: control group; CB: treated group with CB; NAC: treated group with CB and supplemented with NAC. Values are expressed as mean \pm SD ($n = 12$).

^aSignificantly different from the control group ($p < 0.05$).

^bSignificantly different from the CB group ($p < 0.05$).

Figure 1: Effect of NAC on AChE activity in erythrocytes of CB-exposed rats following 30 days exposure.

CTR: control group; CB: treated group with CB; NAC: treated group with CB and supplemented with N-acetylcysteine. Values are expressed as mean \pm SD ($n = 12$). ^aSignificantly different from the control group ($p < 0.05$). ^bSignificantly different from the CB group ($p < 0.05$).

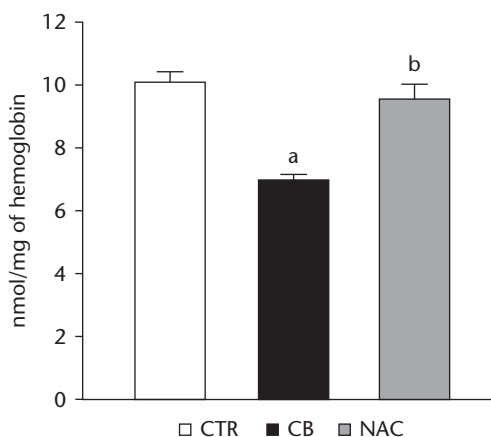
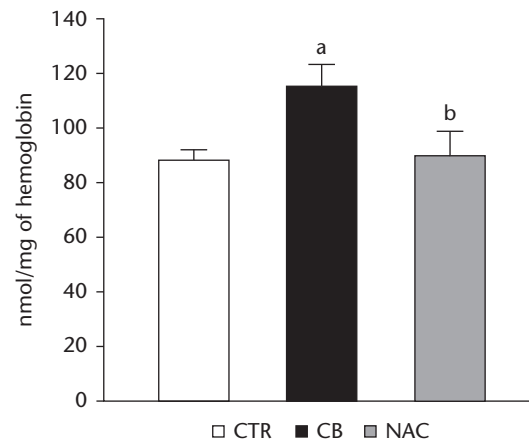
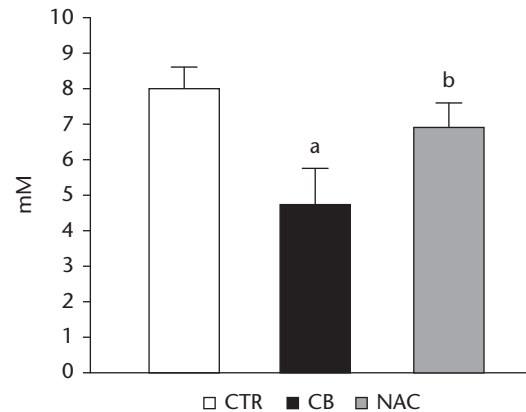


Figure 2: Effect of NAC on MDA activity in erythrocytes of CB-exposed rats following 30 days exposure.

CTR: control group; CB: treated group with CB; NAC: treated group with CB and supplemented with N-acetylcysteine. Values are expressed as mean \pm SD ($n = 12$). ^aSignificantly different from the control group ($p < 0.05$). ^bSignificantly different from the CB group ($p < 0.05$).

**Figure 3: Effect of NAC on TSH content in erythrocytes of CB-exposed rats following 30 days exposure.**

CTR: control group; CB: treated group with CB; NAC: treated group with CB and supplemented with N-acetylcysteine. Values are expressed as mean \pm SD ($n = 12$). ^aSignificantly different from the control group ($p < 0.05$). ^bSignificantly different from the CB group ($p < 0.05$).

**Figure 4: Effect of NAC on GSH level in erythrocytes of CB-exposed rats following 30 days exposure.**

CTR: control group; CB: treated group with CB; NAC: treated group with CB and supplemented with N-acetylcysteine. Values are expressed as mean \pm SD ($n = 12$). ^aSignificantly different from the control group ($p < 0.05$). ^bSignificantly different from the CB group ($p < 0.05$).

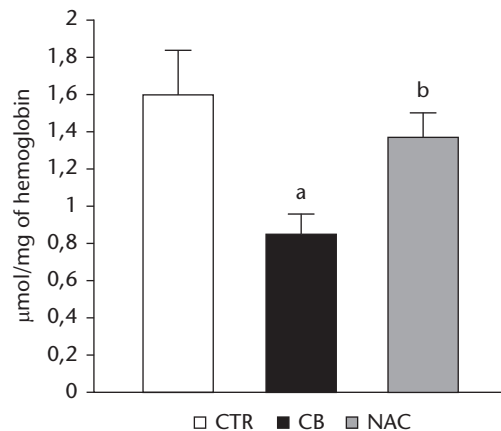


Table 2: Effect of NAC on levels of antioxidant enzymes (SOD and CAT) and on the activity of glutathione peroxidase (GPx) and glutathione S-transferase (GST) in erythrocytes of CB-exposed rats following 30 days exposure.

	CTR	CB	NAC
SOD (U/mg of Hb)	0.49 ± 0.022	0.65 ± 0.0221 ^a	0.48 ± 0.02 ^b
CAT (U/mg of Hb)	0.576 ± 0.115	2.476 ± 0.573 ^a	0.882 ± 0.222 ^b
GPx (nmol oxidized GSH min/mg of Hb)	66.987 ± 5.263	41.658 ± 3.541 ^a	59.485 ± 5.74 ^b
GST (nmol of conjugated CDNB min/mg of Hb)	125.542 ± 7.365	95.234 ± 5.214 ^a	110.1 ± 3.47 ^b

CTR: control group; CB: treated group with CB; NAC: treated group with CB and supplemented with NAC. Values are expressed as mean ± SD ($n = 12$).

^aSignificantly different from the control group ($p < 0.05$).

^bSignificantly different from the CB group ($p < 0.05$).

3.3.2. GSH and total thiol content

GSH and total thiol contents of various groups are depicted in Figures 3 and 4. In case of CB-treated animals, there was a significant decrease in the GSH content (88.23%) as compared to the control group. NAC treatment protected against CB-induced reduction in GSH levels (162.35%) in the rat erythrocyte as evident by an increase in GSH content. Total thiol content was significantly decreased in the erythrocyte of CB treated animals (66.87%) as compared to the control animals. NAC treatment to the CB-exposed animals significantly increased the TSH content (143.75%) as compared to the CB-treated animals.

3.3.3. Antioxidant enzymes

The effects of CB and NAC co-administration on the activity of the antioxidant enzymes are reported in Table 2. CB induced a significant ($p < 0.05$) increase in SOD and CAT activities versus a decrease in GPx and GST activities. Nevertheless, NAC, supplemented in drinking water, caused a dramatic decrease in SOD and CAT activities and an increase in GPx and GST activities of rats supplemented with NAC until CB treated values.

4. DISCUSSION

The uncontrolled exposure to toxic pesticides, such as CB, is capable of inflicting biological damage leading to the pathology of many conditions including blood disorders. In the present investigation, administration of CB caused significant alterations in some hematological parameters. The significant alteration in the number of RBC, Hb, hematocrit, and reticulocytes in this experimental model indicates that exposure to CB could lead to bleeding anemia. Our findings on CB treatment-related hematological toxicity are in agreement with the report on rats exposed to CB (43 mg/kg) (Kandil *et al.*, 2006). The co-administration with NAC significantly improved hematological parameters by restoring to normalcy the RBC number, Hb level, and reticulocytes percentage. Our results corroborate with those of Cuzzocrea *et al.* (2001), they showed that NAC, *in vivo*, NAC significantly prevent RBC alteration and prevent the loss of Hb content.

It is well-known that AChE is irreversibly inhibited by carbamates insecticides by carbamylation (O'Malley, 1997). Once a given molecule of cholinesterase has been inhibited reversibly, the only way of replacing the activity is through synthesis of new enzyme. The synthesis does not occur in circulating RBCs and only way RBC-AChE is renewed in the blood is through synthesis of enzyme in erythropoietic cells of the bone-marrow and its subsequent entry into the circulating blood (Abdollahi *et al.*, 1999; Gordon and Rowsey, 1998). Therefore, measurement of erythrocyte AChE activity can be a marker of chronic exposure to carbamates pesticides anti-AChEs (O'Malley, 1997). Our results revealed that CB alone caused a statistically significant decrease (43.78%) in the activity of AChE in erythrocytes of male rats compared to the control results (Figure 1). The treatment with NAC increases AChE activity. Also, when AChE is reversibly inhibited in erythrocytes, the recovery toward normal values depends on new cell entering the bloodstream, and has been calculated for most organophosphorus pesticides to correspond to 1%/day (Gordon and Rowsey, 1998).

LPO has been suggested as one of the molecular mechanisms involved in organophosphorus and carbamate pesticide-induced toxicity (Kehrer, 1993). Malondialdehyde (MDA) level in CB treatment was significantly ($p \leq 0.05$) higher than that in control group and the change accounted to 30.9% in male rats (Figure 2). These indirectly suggest an increased production of oxygen free radicals in rats. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a LPO product. Carbamates pesticides have been reported to induce oxidative stress, as shown by enhanced MDA production (Amer *et al.*, 2002; Eraslan *et al.*, 2009; Mansour *et al.*, 2009; Rai *et al.*, 2009). The use of NAC in conjunction with CB affected such elevation in the level of MDA; bringing it within the normal limits ($p \leq 0.05-0.01$). The normalization

of LPO following NAC treatment is very likely due to its antiperoxidative properties, as has been shown previously by Zafarullah *et al.* (2003) and Thorin-Trescases *et al.* (2010). NAC plays an important role in regulation of cellular GSH that is vital to cellular antioxidant defense (Lucht *et al.*, 1987). It has also been reported to interact with cell membranes to stabilize them against various damaging effects, including those due to oxidative injuries (Zhang *et al.*, 1990). Also, GSH-dependent defense against xenobiotics is a multifaceted phenomenon that has been well characterized in mammals. CB exposure decreased GSH content in the erythrocyte. Our findings are in accordance with those of Soderpalm-Berndes and Onfelt (1988), who have reported lowering of GSH levels by carbaryl. The mechanism involved in GSH depletion after carbamate exposure involves carbamylation of TSH (Ningaraj *et al.*, 1998). The decrease in GSH levels might diminish the overall antioxidant potential of the erythrocyte resulting in increased LPO following CB exposure. GSH depletion may also effect the activation and translocation of transcription factors like NF- κ B and c-Jun/activating transcription factor (Rokutan *et al.*, 1998). NAC administration was seen to augment GSH levels and confer its protective effects in CB hematotoxicity. The decreased TSH content on CB exposure rats might be contributed by reduction in GSH levels and/or could be due to decrease in protein thiol groups.

Elevation in the level of MDA, aftereffect of CB administration, result in dysfunction and the question of whether oxidative stress is a major cause of erythrocyte injury remains equivocal. However, the antioxidant enzymes SOD, GST, and CAT limit the effects of oxidant molecules on tissues and are active in the defense against oxidative cell injury by means of their being free radical scavengers (Kyle *et al.*, 1987). These enzymes work together to eliminate active oxygen species and small deviations in physiological concentrations may have a dramatic effect on the resistance of cellular lipids, proteins, and DNA to oxidative damage. The enzymatic antioxidants in erythrocytes may counteract oxidative stress. For instance, SOD catalyzes the conversion of superoxide radical to hydrogen peroxide, while CAT converts hydrogen peroxide to water. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS (Mates and Sanchez-Jimenez, 1999a; Mates *et al.*, 1999b). Moreover, the existence of a mutually supportive relationship between metalloenzyme SOD, which accelerates the dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 , and CAT, which converts the deleterious peroxide radicals into water and oxygen, provides the first line of defense to the cells (Adedara and Farombi, 2010). The increased activities of erythrocyte SOD and CAT observed in CB-treated animals may lead to decreased steady-state levels of the deleterious superoxide radicals and H_2O_2 .

In fact, hydroxyl radical could be formed by the interaction of superoxide radical with H_2O_2 through the Haber–Weiss reaction (Adedara *et al.*, 2013). The overwhelming generation of free radicals in the blood milieu may contribute to the inactivation of these enzymes and may increase the oxidative stress in erythrocyte of animals treated with CB. These findings were in line with the previous reports, showing a decrease in free radical scavenger enzyme activities in rats exposed to carbofuran, principal metabolite of CB (Mansour *et al.*, 2009; Rai *et al.*, 2009). However, we found a decrease in the GPx and GST activities in CB-treated groups (Table 2). These data could be explained by an increase in ROS formation and oxidative stress induction. This finding is not rare. Indeed, similar results were observed in rats treated with carbamate pesticide (Fukuto, 1990; Abdollahi *et al.*, 2004; Mansour *et al.*, 2009).

However, amelioration was evident by marked increases in the SOD and CAT activities in erythrocyte of animals co-treated with NAC in comparison with those of CB-treated animals. As with other antioxidant enzymes, NAC was found to protect GPx from carbofuran. Farbiszewski *et al.* have also found protective effect of NAC on GPx activity in methanol intoxicated rats (Farbiszewski *et al.*, 2000). The present results may indicate that there was a quick scavenging of superoxide and hydroxyl radicals by NAC, possibly due to its high bioavailability, to keep their normal levels, thereby allowing the erythrocyte antioxidant system to efficiently decrease CB-mediated erythrocyte ROS generation. The ability of NAC to inhibit hydroxyl and superoxide anion radicals *in vitro* has been reported by Zachwieja *et al.* (2005).

In summary, severe oxidative stress can lead to hemolysis. In principle, the erythrocyte is very sensitive to peroxidative reactions. Under normal conditions, the erythrocyte is very well protected against peroxidative reactions by the presence of CAT and GSH. This balance, however, can be disturbed by different chemicals. Oxidative damage can result indirect injury to the cell membrane due to LPO and bring about changes in membrane proteins (i.e., enzymes) and consequently, the permeability of such membranes of any erythrocyte (Brkić *et al.*, 2008). CB caused erythrope-
nia and a reduction in the Ht and Hb levels. These results suggest existing of absolute anemia as a result of hemolytic or depression anemia (Jain, 1993). Surprisingly, the supplementation of NAC effectively attenuated the alterations induced by CB in the hematological variables and thereby protecting the heme from CB induced oxidative stress. CB might reduce the oxidative stress by scavenging the free radical and ROS by its effective antioxidative and free radical scavenging activities reported by El-bini Dhouib *et al.* (2014).

In mechanistic term, the presence of acetyl and sulfhydryl groups makes NAC a potent inhibitor of LPO. In addition, NAC is a thiol, a mucolytic agent, and a precursor of L-cysteine and reduced GSH. NAC is a source of sulfhydryl groups in cells and scavenger of free radicals as it interacts with ROS such as OH and H_2O_2 (Aruoma *et al.*, 1989). GSH is currently one of the most studied antioxidants as it is endogenously synthesized basically in all cells. Among many, established roles for GSH are the following: (i) antioxidant defense, (ii) detoxification of electrophilic xenobiotics, (iii) modulation of redox (oxidation–reduction reaction)-regulated signal transduction, and (iv) storage and transport of cystein. GSH has an important role in maintaining the redox state of the cell (Kerksick and Willoughby, 2005).

It thereby exerts a profound protective effect on cells. Of the three amino acids in the GSH structure (glutamate, glycine, and cysteine), cysteine has the lowest intracellular concentration (Aruoma *et al.*, 1989). Cysteine availability can limit the rate of GSH synthesis during times of oxidative stress. NAC is an acetylated cysteine residue able to increase cell protection to oxidative stress. NAC is an effective scavenger of free radicals as well as a major contributor to maintenance of the cellular GSH status. NAC can minimize the oxidative effect of ROS through correcting or preventing GSH depletion (Kerksick and Willoughby, 2005).

Taken together, the data presented in this study clearly demonstrate, for the first time, that both NAC elicited significant protection against CB-mediated hematotoxicity. Moreover, NAC is efficient in protecting the erythrocyte from toxicity and oxidative damage induced by CB as evidenced by the restoration of antioxidant status and biomarkers of erythrocyte damage to normalcy. Also, NAC may be potential therapeutic tools for hematotoxicity resulting from CB exposure.

5. CONCLUSION

In view of the data of the present study, it can be concluded that CB-induced oxidative stress and LPO in erythrocytes of male rats and conjunction supplementation of NAC has ameliorated these effects. The precise mechanism of the observed protective effect of NAC cannot be ascertained from the results of this study and thus remains to be explored in future.

Author Contributions

All authors contributed equally to this work.

References

- Abdollahi M, Jalali N, Sabzevari O (1999). Pesticide poisoning during an 18-month period (1995–1997) in Tehran, Iran. *Iranian Journal of Medical Science*, 24: 77-81.
- Abdollahi M, Balali-Mood M, Akhgari M (1996). A survey of cholinesterase activity in healthy and organophosphate-exposed populations. *Iranian Journal of Medical Science*, 21: 63-66.
- Abdollahi M, Ranjbar A, Shadnia S, *et al.* (2004). Pesticides and oxidative stress: A review. *Medical Science Monitor*, 6: 141-147.
- Adedara IA, Farombi EO (2010). Induction of oxidative damage in the testes and spermatozoa and hematotoxicity in rats exposed to multiple doses of ethylene glycol monoethyl ether. *Human & Experimental Toxicology*, 29: 801-812.
- Adedara IA, Ebokaiwe AP, Farombi EO (2013). Tissues distribution of heavy metals and erythrocytes antioxidant status in rats exposed to Nigerian bonny light crude oil. *Toxicology and Industrial Health*, 29: 162-168.
- Aebi HE (1984). Catalase *in vitro*. *Methods in Enzymology*, 105: 121-126.
- Amer M, Metwalli M, El-Magd YA (2002). Skin diseases and enzymatic antioxidant activity among workers exposed to pesticides. *Eastern Mediterranean Health Journal*, 8: 201-211.
- Aruoma OI, Halliwell B, Hoey BM (1989). The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radical Biology & Medicine*, 6: 593-597.
- Atkuri KR, Mantovani JJ, Herzenberg LA (2007). N-acetylcysteine – A safe antidote for cysteine/glutathione deficiency. *Current Opinion in Pharmacology*, 7(4): 355-359.
- Begue JA, Aust SD (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, 52: 302-310.
- Brkić DV, Vitorović SL, Gašić SM, *et al.* (2008). Carbofuran in water: Subchronic toxicity to rats. *Environmental Toxicology and Pharmacology*, 25(3): 334-341.
- Capkin E, Boran H, Altinok I (2014). Response of acetylcholinesterase (AChE) in the erythrocyte and liver of rainbow trout exposed to carbosulfan. *Turkish Journal of Fisheries and Aquatic Sciences*, 14: 643-650.
- Cuzzocrea S, Riley DP, Caputi AP, *et al.* (2001). Antioxidant therapy: A new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacological Reviews*, 53: 135-159.
- Dacie JV, Lewis SM (1968). *Practical Hematology*, 4th Ed., Churchill, London.
- El-Bini Dhoubi I, Lasram MM, Abdeladhim M, *et al.* (2014). Immunosuppression and oxidative stress induced by subchronic exposure to carbosulfan in rat spleen: Immunomodulatory and antioxidant role of N-acetylcysteine. *Toxicology Mechanisms and Methods*, 24(6): 417-427.
- Ellman GL, Courtney KD, Andres VJ, *et al.* (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7: 88-95.
- El Midaoui A, Ismael MA, Lu H (2008). Comparative effects of Nacetyl-L-cysteine and ramipril on arterial hypertension, insulin resistance, and oxidative stress in chronically glucose-fed rats. *Canadian Journal of Physiology and Pharmacology*, 86: 752-760.
- Eraslan G, Kanbur M, Silici S (2009). Effect of carbaryl on some biochemical changes in rats: The ameliorative effect of bee pollen. *Food and Chemical Toxicology*, 47(1): 86-91.

- Farbiszewski R, Witek A, Skrzydlewska E (2000). N-acetylcysteine or trolox derivative mitigate the toxic effects of methanol on the antioxidant system of rat brain. *Toxicology*, 156: 47-55.
- Fukuto TR (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Environmental Health Perspectives*, 87: 245-254.
- Gordon CJ, Rowsey PJ (1998). Poisons and fever. *Clinical and Experimental Pharmacology and Physiology*, 25: 145-149.
- Habig WH, Pabst MJ, Jakoby WB (1974). The glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249: 7130-7139.
- Hu ML, Dillard CJ (1994). Plasma SH and GSH measurement. *Methods in Enzymology*, 233: 385-387.
- Kandil MA, El-Kashoury AA, El-Said MM, *et al.* (2006). Interactive effects of imidacloprid, profenofos and carbosulfan at low concentrations on homeostasis and haematological indices in male albino rats. *Journal of Egyptian Society of Toxicology*, 35: 69-78.
- Kehrer JP (1993). Free radicals as mediator of tissue injury and disease. *Critical Reviews in Toxicology*, 23(1): 21-48.
- Kerksick C, Willoughby D (2005). The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*, 2: 38-44.
- Kyle ME, Miccadei S, Nakae D, *et al.* Superoxide dismutase and catalase protect cultured hepatocytes from the cytotoxicity of acetaminophen. *Biochemical and Biophysical Research Communications*, 149: 889-896.
- Lucht WD, Englis DK, Bernard GR (1987). Prevention of release of granulocyte aggregates into sheep lung lymph following endotoxemia by N-acetylcysteine. *The American Journal of Medical Sciences*, 294: 161-167.
- Mansour SA, Mossa AH, Heikal TM (2009). Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: *In vitro* studies. *Toxicology and Industrial Health*, 25: 557-563.
- Mates JM, Sanchez-Jimenez F (1999a). Antioxidant enzymes and their implications in pathophysiologic processes. *Frontiers in Biosciences*, 4: 339-345.
- Mates JM, Perez-Gomez C, Nunez DCI (1999b). Antioxidant enzymes and human diseases. *Clinical Biochemistry*, 32: 595-603.
- McCord JM, Fridovich I (1969). Superoxide dismutase an enzymatic function for erythrocyte hemoglobin. *The Journal of Biological Chemistry*, 244: 6049-6055.
- Ningaraj NS, Schloss JV, Williams TD, *et al.* (1998). Glutathione carbamylation with S-methyl-N,N-diethylthiolcarbamate sulfoxide and sulfone: Mitochondrial low Km aldehyde dehydrogenase inhibition and implications for its alcohol deterrent action. *Biochemical Pharmacology*, 55: 749-756.
- O'Malley M (1997). Clinical evaluation of pesticide exposure and poisonings. *Lancet*, 349: 1161-1166.
- Ortolani O, Conti A, De Gaudio AR (2000). The effect of glutathione and N-acetylcysteine on lipoperoxidative damage in patients with early septic shock. *American Journal of Respiratory and Critical Care Medicine*, 161: 1907-1911.
- Rai DK, Rai PK, Rizvi SI (2009). Carbofuran-induced toxicity in rats: Protective role of vitamin C. *Experimental and Toxicologic Pathology*, 61: 531-535.
- Rokutan K, Teshima S, Miyoshi M (1998). Glutathione depletion inhibits oxidant induced activation of nuclear factor-kappa B, AP-1, and c-Jun/ATF-2 in cultured guinea-pig gastric epithelial cells. *Journal of Gastroenterology*, 33: 646-655.
- Rushworth GF, Megson IL (2014). Existing and potential therapeutic uses for N-acetylcysteine: The need for conversion to intracellular glutathione for antioxidant benefits. *Pharmacology & Therapeutics*, 141(2): 150-159.
- Soderpalm-Berndes C, Onfelt A (1998). The action of carbaryl and its metabolite alpha-naphthol on mitosis in V79 Chinese hamster fibroblasts: Indications of the involvement of some cholinesterase in cell division. *Mutation Research*, 201: 349-363.
- Tietze F (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. *Analytical Biochemistry*, 27: 502-522.
- Thorin-Trescases N, Voghel G, Farhat N (2010). Âge et stress oxydant Vers un déséquilibre irréversible de l'homéostasie endothéliale. *Medical Sciences*, 26: 875-880.
- Umetsu N, Fukuto RT (1982). Alteration of carbosulfan [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-n-butylaminosulfonyl) methylcarbamate] in the rat stomach. *Journal of Agricultural and Food Chemistry*, 30: 555.
- Wendel A (1981). Glutathione peroxidase. *Methods in Enzymology*, 77: 325-333.
- Zachwieja J, Zaniew M, Bobkowski W (2005). Beneficial in vitro effect of N-acetyl-cysteine on oxidative stress and apoptosis. *Pediatric Nephrology*, 20: 725-731.
- Zafarullah M, Li WQ, Sylvester J (2003). Molecular mechanisms of N-acetylcysteine actions. *Cellular and Molecular Life Sciences*, 60: 6-20.
- Zhang Y, Marcillat O, Giulivi C (1990). The oxidative inactivation of mitochondrial electron-transport chain components and ATPase. *The Journal of Biological Chemistry*, 265: 16330-16336.

Citation: El-Bini Dhouib I, Annabi A, Jrad A, *et al.* (2015). Protective Effect of N-Acetylcysteine Against Toxicity on the Rat Blood After Chronic Exposure to Carbosulfan. *Recent Advances in Biology and Medicine*, 1: 18-25.

Source of Support: None; **Conflict of Interest:** None.