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Original article

Antioxidant, histopathological and biochemical outcomes of short-term exposure to acetamiprid in liver and brain of rat: The protective role of N-acetylcysteine and S-methylcysteine

Nazanin Khovarnagh, Bagher Seyedalipour *

Department of Molecular and Cell Biology, Faculty of Basic Science. University of Mazandaran, Babolsar, Iran

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ABSTRACT

The present study was conducted to investigate the protective effects of N-Acetyl-L-cysteine (NAC) and S-methyl- L-cysteine (SMC) against hepatic oxidative stress and brain damage induced by acetamiprid (ACP) in rats, which were evaluated by histopathological changes, measuring serum biomarkers and antioxidant defense systems. In this study, 42 rats were randomly divided into 6 groups and administered by intraperitoneally for one week: the control group, the sham group (normal saline), ACP alone (5 mg/kg) (group1), NAC alone (160 mg/kg) (group2), ACP + SMC (100 mg/kg) (group3), ACP + NAC (group 4) and ACP + NAC + SMC (group 5). Our results showed that acetamiprid induces liver injures including infiltration of inflammatory cells, congestion and altered histo-architecture and brain damages including gliosis, hyperemia and necrosis. The biochemical analyses showed that acetamiprid significantly altered the structural and biochemical profiles of liver which may be due to the loss of integrity of cell membranes. Furthermore, antioxidant parameters results of ACP group revealed that glutathione (GSH) and total antioxidant capacity (TAC) levels decreased significantly, while lipid peroxidation (LPO) content and glutathione-S-transferase (GST) and catalase (CAT) activities increased in both tissues ($P < 0.05$), suggesting tissue oxidative damage, which was also confirmed histopathological. Conversely, administration of NAC and SMC ameliorated LPO, GSH content and antioxidant enzymes system considerably ($P < 0.05$) in both tissues. Moreover, NAC and SMC administration also improved liver and brain malfunction. These results indicate that both NAC and in to a lesser amount SMC have a potent antioxidant protection in both tissues of rat against ACP-induced oxidative stress.

1. Introduction

Pesticides play an important role in the control of harmful insects, thus are widely used in agriculture and cause environmental pollution and also potential hazards to human health (Speck-Planche et al., 2012). The neonicotinoids are belonging to wide families which include acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam (Pisa et al., 2015). Neonicotinoids, the newest group of insecticides, have remarkable and systemic influence on protecting crops against pests (Mondal et al., 2009; Pisa et al., 2015; Wood and Goulson, 2017). Several studies have demonstrated that lipid peroxidation and their biological effects through electrophilic attack on hepatic and brain tissues are induced by neonicotinoids in rats and humans (Sharma et al., 2005). Acetamiprid is an organic compound with IUPAC (the International Union of Pure and Applied Chemistry) name N-[(6-chloro-3-pyridyl) methyl]-N'-cyano-N-methyl-acetamidine and comprising a heterocyclic ring (the 6-chloro-3-pyridylmethyl moiety), which is one of the most effective insecticides for the protection of crops in the world (Sanyal et al., 2008). ACP is highly soluble in water and highly permeable to soil and potentially toxic to humans. Inhaling it can cause symptoms such as headache, dizziness, vertigo, nausea and vomiting (Todani et al., 2008). Acetamiprid plays an important role in the induction of oxidative stress and increased oxidants by producing reactive oxygen species (ROS) (Zhang et al., 2011). Thus, the investigation of antioxidant

* Corresponding author.
E-mail address: b.seyedalipour@umz.ac.ir (B. Seyedalipour).

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defenses and oxidative stress biomarkers can be very useful to study oxidative damage in tissues and health implications.

In natural conditions, there is a balance between the production and elimination of free radicals, which causes serious damage to the cell (Mansour and Mossa, 2010). Most cells can endure a mild percentage of oxidative stress because they have sufficient repair and antioxidant defense capacity to delay or remove cellular damage. Excessive free radicals can cause lipid peroxidation, DNA damage, and depletion of antioxidant reserves (Poljsak et al., 2013). Thus, the measurement of antioxidant status can be estimated by endogenous enzymatic and nonenzymatic antioxidant defense systems to eliminate ROS.

N-Acetyl-L-cysteine (NAC), an amino acid-derived compound of L-cysteine, which is an excellent source for the production of a sulfide group. NAC as an antioxidant can ameliorate oxidative stress-induced damage. This protective property can be both directly by neutralizing free radicals and indirectly by stimulate glutathione production (Aruoma et al., 1989). Thus, NAC is a rich source of sulfhydryl groups and have an important role in stimulating glutathione synthesis, detoxification and elimination of free radicals (Kerksick and Willoughby, 2005). S-methyl-Lcysteine (SMC) is a water-soluble organosulfur compound that found in a variety of plants, such as *Aloe vera*, *Allium sativum*, *Carina papaya* (Iciek et al., 2009). SMC is one of the amino acid derivatives of L-cysteine has the ability to eliminate free radicals due to the presence of thiol groups in their structure (Kumari and Augusti, 2002). Many studies have revealed that SMC as an antioxidant has a role in antioxidant responses against pro-oxidants and preventing liver cancer (Thomas et al., 2015). Thus, SMC and NAC, as a hydrophilic antioxidant, are reported to neutralize reactive oxygen species (ROS), a scavenger of hydrogen Peroxide, protect against free radical and also act as pro-oxidants in humans during severe and acute inflammatory. The objective of this study was conducted to investigate the antioxidative effects of NAC and SMC against oxidative stress and also assay of activity glutathione-S-transferase, catalase and levels of total antioxidant capacity, estimation of glutathione and malondialdehyde in brain and liver of rats. Hence, we peruse the protective effect of NAC and SMC following treatment with acetamiprid in rat.

2. Materials and methods

2.1. Materials

Thiobarbituric acid (TBA), sodium acetate, di-sodium hydrogenphosphate, hydrogen peroxide (H2O2), Ethylene diamine tetra acetic acid (EDTA), trichloroacetic acid (TCA), 1-chloro-2,4-dinitrobenzene (CDNB), and o-dianisidine dihydrochloride were obtained from Merck maximum Reduced glutathione (GSH) and 2,4,6-tris(2pyridyl)-s-triazine (TPTZ) were also purchased from Sigma Company. All utilized reagents were of analytical grade and greatest purity.

2.2. Experimental design and animal treatments

In this experimental study, forty-two adult male Wistar rats with weight 190 ± 30 g were purchased from Pasteur Institute. Animals were kept in polyethylene cages with adequate food and water and under the controlled temperature (24 ± 2 °C), humidity (55 ± 5%) and normal light/dark cycle during all phases of experiments. This study was carried out in agreement with the Guide of Care and were approved by the ethics committee on animal experimentation of the University of Mazandaran. To design of experiment, forty-two rats were randomly divided into seven groups with six rats in each group. The injection was done intraperitoneally for one week. The value of LD_{50} (the amount of a material which causes the death of 50% (one half) of a group of test animals) for ACP dose was selected on the basis of previously reported (Chakroun et al., 2016 18). We determined dose of the experimental groups less than the LD_{50} of ACP. The groups were divided as follows: Control group fed with a commercial feed and adequate water while sham group was intraperitoneally (i.p) injected with 0.5 ml normal saline solution (daily, one week). All experimental groups were injected daily intraperitoneally using a syringe for one week. Experimental group 1(ACP) was received 5 mg/kg ACP solution dissolved in normal saline. Experimental group 2 (NAC) was received 160 mg/kg NAC dissolved in normal saline. Experimental group 3(ACP + SMC), simultaneously; received 5 mg/kg ACP and 100 mg/kg SMC. Experimental group 4 (ACP + NAC), simultaneously; received 5 mg/kg ACP and 160 mg / kg NAC. Experimental Group 5(ACP + NAC + SMC), simultaneously; received 5 mg/kg ACP, 160 mg/kg NAC and 100 mg / kg SMC.

At the end of experimental period, animals were fasted overnight (12 h), anesthetized and blood samples were taken from the heart. The blood sample was centrifuged at 3000 rpm for 15 min to separate the serum from the clot and then stored at −20 °C for biochemical analysis. To confirm the liver function and injury, serum biochemical analysis was carried out to determine activities of aspartate transaminase (AST) (Pars Azmoon Lot no, 94006), alanine transaminase (ALT) (Pars Azmoon Lot no, 94003), alkaline phosphatase (ALP) (Pars Azmoon Lot no, 94009), lactate dehydrogenase (LDH) (Pars Azmoon Lot no, 94005), Total protein (TP) (Pars Azmoon Lot no, 94004) and Albumin (Pars Azmoon Lot no, 94002) using autoanalyzer.

To prepare homogenate and histological analysis, rats were sacrificed, liver and brain tissues were excised, washed in normal saline, weighed and each one was divided into two parts. The first part was sliced and fixed immediately in formalin buffer and finally dehydrated with graded ethanol (80–100%). Tissue samples were embedded in paraffin and subsequently, the tissues were sliced into 5 μm sections using microtome. For histopathological evaluation, staining was performed with hematoxylin and eosin (H&E). Then, for observation of cellular damages, tissue samples were evaluated with light microscope. To determine an appropriate scoring system for liver and brain tissue changes, the scores were derived semi-quantitatively using light microscopy and scored in four categories based on the intensity of alterations: 0, absent; 1, mild; 2, moderate; 3 severe.

The second part was washed and homogenized in buffer 0.1 M sodium phosphate (pH 7.4) at a ratio of 1: 5 using Teflon glass homogenizer. The homogenate was centrifuged (12000 rpm at 4 °C for 20 min) in a refrigerated centrifuge and supernatant was collected and stored at −20 °C and used as sample for antioxidant assays.

2.3. Oxidative stress biomarkers

2.3.1. Measurement of catalase activity

Catalase activity was determined in liver and brain homogenate according to method of Aebi (1984) by measuring of the decomposition of H_2O_2 (Aebi, 1984). Briefly, 10 μL of diluted homogenate was added in the quartz cuvette containing 2 ml sodium phosphate buffer (50 mM, pH 7) and 1 ml of H_2O_2 (30 mM). Catalase activity was calculated by determining alteration absorbance at 240 nm for 2 min using extinction coefficient of 39.4 M^{-1} cm^{-1} and expressed as U/g tissue for brain and liver. One unit of CAT is the amount of enzyme that decomposes 1.0 μmol of H_2O_2 per min at pH 7.0 and 25 °C.

2.3.2. Determination of lipid peroxidation

Measurement of Malondialdehyde Level (MDA) perform by the method of Buege and Aust (1978). Briefly, 100 μL of tissue homogenates were mixed with 2 ml of thiobarbituric acid (TBA) reagent (15% TCA, 0.375% TBA and 0.25 N HCl). Reaction mixture was incubated in water bath (100 °C) for 15 min. Afterwards, the mixture was cooled and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm at room temperature against the blank. MDA Level was calculated using extinction coefficient 155 $mM^{-1}cm^{-1}$ and expressed as nmol/ g tissue for brain and liver.

2.3.3. Estimation of GSH

The level of glutathione was determined by the methods of sedlak and Lindsay (1968). Briefly, 100 μL of tissue homogenates were mixed with 4.4 ml of 10 mM EDTA and 500 μL of TCA (10%). Mixtures were centrifuged at 10000 rpm for 3 min, then supernatant was collected and mixed with 50 μL of DTNB (10 mM) and absorbance of products read at 412 nm. Standard curve was prepared using pure reduced glutathione in the range of 12.5–200 μM. Results were expressed as μM/g tissue for liver and brain tissue.

2.3.4. Assay of Glutathione-S-transferase

GST activity was determined according to the procedure of Habig et al. (1974). Briefly, 3 ml reaction mixture consisted of 2.850 ml of phosphate buffer (100 mM, pH 6.5), 50 μL of GSH (60 mM), 50 μL of CDNB (60 mM, prepared in ethanol) and 50 μL of sample. Changes of absorbance at 340 nm for 3 min were recorded. GST activity was calculated using extinction coefficient of 9.6 mM^{-1}/cm^{-1} for CDNB-GSH conjugate and expressed as U/g for liver and brain tissues.

2.3.5. Measurement of total antioxidant capacity (TAC)

Total antioxidant capacity was measured as described by Benzie and Strain (1999). Briefly, a working solution of FRAP (ferric reducing antioxidant power) was prepared by mixing 25 ml of 0.3 M acetate buffer (pH 3.6) with 2.5 ml of 10 mM TPTZ solution in HCl and 2.5 ml of 20 mM $FeCl_3$. 6 H_2O in a 10:1:1 ratio. Then, to 1.5 ml of the FRAP reagent was added 50 μL of sample and incubated for 10 min at 37 °C. The absorbance of each sample solution was measured at 593 nm against the blank. For the calibration curve, $FeSO_4$ was prepared in FRAP reagent in the range of 50–1000 μM. The results were expressed as μM/ g of tissue.

2.4. Statistical analysis

Data were analyzed using SPSS version 21 statistical software. To compare responses between groups, a one-way analysis of variance (ANOVA) and Tukey posttest were performed for antioxidant parameters. Quantitative data were expressed by mean ± SD. A P value less than 0.05 were considered significant. To study significant differences in the mean scores of histopathologic lesions, statistical analysis was performed using the Kruskal Wallis test and Paired comparisons were performed with Mann-Whitney *U* test. A P value of $\leq$0.05 was considered significant.

3. Results

3.1. Biochemical analysis results

The mean ± SD values of serum ALT, AST, ALP, LDH, albumin and total protein levels among the studied groups were presented in (Table 1). Statistical analysis showed that serum ALT ($p < 0.001$), ALP ($p < 0.001$), AST ($p < 0.05$), albumin ($p < 0.01$) and total protein ($p < 0.001$) levels (excluding LDH) had a significant difference in the studied groups. The ALT activity in the control group was 27.50 ± 1.76 U/l. Comparison of means of ALT activity in different treatment groups had shown no significant differences between control and sham. The mean activity of ALT was increased in serum after ACP administration (44.58 ± 3.07, $p < 0.001$) in comparison with the control group (27.50 ± 1.76 U/l; Table 1). Administration NAC and SMC with ACP significantly improved ALT level in experimental group 3 (P = 0.028), group 4 (P = 0.004) and group 5 (P = 0.05) in compared to control group. Simultaneous administration of ACP with NAC and SMC in experimental group 5 (P = 0.04) significantly decreased ALT level in serum when compared to ACP group.

The AST activity in the control group was 111.41 ± 5.17 U/l. Exposure to ACP in experimental group 1 (158.83 ± 14.03, $p < 0.05$) significantly increased ALT level compared to control group. Simultaneous administration of ACP with NAC and SMC in experimental group 3, group 4 and group 5 had shown no significant differences in AST level in comparison with group 1(ACP), but a noticeable decrease was observed. The ALP activity in the control group was 234.66 ± 33.01 U/l. ACP cause significant increase in the ALP serum levels (556.8 ± 21.14 U/l, $p < 0.001$) in comparison with the control group (234.66 ± 33.01 U/l). Simultaneous administration of ACP with NAC and SMC in experimental group 4 (P = 0.008) and group 5 (P = 0.047) significant decreased AST level in serum when compared to group 1(ACP). The results of the statistical analyses with ANOVA had revealed no significant differences in serum LDH levels in the studied groups. The LDH activity in the control group was 1285.83 ± 146.99 U/l. The mean activity of LDH was increased in serum after administration of ACP (1628.33 ± 147.22U/l, $p > 0.05$) in comparison with the control group, but no significant (Table 1). In ACP + NAC, ACP + SMC, ACP + NAC + SMC groups, there was a slight decrease in activity of LDH in comparison with ACP group, but no significant.

The means of albumin and total protein levels in different groups of rats are demonstrated in Table 1. The albumin and total protein levels in the control group were 2.94 ± 0.05 g/dl and 5.94 ± 0.10 g/dl, respectively. Albumin content had shown no significant differences between control, sham and experimental groups 2(NAC). A significant decrease in the albumin level of serum was observed following exposure to ACP (2.46 ± 0.03 g/dl, $p < 0.001$) in comparison with control. Administration NAC and SMC with ACP significantly improved albumin level in experimental group 3 (P = 0.003), group 4 (P = 0.001) and group 5 (P = 0.017) in compared to control group. Other groups had shown no significant differences in albumin level with ACP group. A more significant decrease was observed in total protein levels of serum (4.99 ± 0.0 1 g/dl, $p < 0.001$) of rat exposure to ACP than that of the control group. Administration NAC and SMC with ACP significantly improved total protein level in experimental group 3 (P = 0.001), group 4 (P = 0.021) and group 5 (P = 0.005) in compared to control group.

3.2. Antioxidant parameters results of liver

To investigate whether the supplements did lead to increased total antioxidant capacity of liver, total antioxidant level was measured (Table 2). Statistical analysis revealed that total antioxidant capacity of liver had a significant difference in the studied groups ($p < 0.001$). Total antioxidant capacity in the liver of the control group was 98.61 ± 10.18 μM. The results of multiple comparisons suggested a significant decrease in the total antioxidant capacity in the ACP group (42.56 ± 5.60 μM/g tissue, $p < 0.001$) in comparison with control group (98.61 ± 10.18 μM/g tissue). Simultaneous administration of ACP with NAC and SMC significantly improved in experimental group 3(P = 0.002), 4 (P = 0.043) and group 5

Table 1
Effect ACP on serum enzyme levels, albumin and total protein of rats treated with NAC and SMC.

	Enzymes					
Groups	ALP(U/L)	AST(U/L)	ALT(U/L)	LDH(U/L)	Alb (g/dl)	TP(g/dl)
Control	234.66 ± 33.01	111.41 ± 5.17	27.50 ± 1.76	1285.83 ± 146.99	2.94 ± 0.05	5.98 ± 0.10
Sham	270.16 ± 20.69^{+++}	117.00 ± 8.36	28.75 ± 6.22^{+++}	1270.66 ± 54.58	2.76 ± 0.09^{+}	5.61 ± 0.15^{+}
G1 (ACP)	556.8 ± 21.14***	158.83 ± 14.03*	44.58 ± 3.07***	1628.33 ± 147.22	2.46 ± 0.03***	4.99 ± 0.1***
G2 (NAC)	344.20 ± 20.14^{++}	119.40 ± 3.61	31.40 ± 5.04^{+}	1149.40 ± 92.51	2.69 ± 0.09	5.62 ± 0.1^{+}
G3 (ACP + SMC)	448.33 ± 38.00**	138.66 ± 9.71	36.65 ± 5.71*	1386.83 ± 109.61	2.56 ± 0.08*	5.19 ± 0.09**
G4 (ACP + NAC)	362.16 ± 57.48^{++}	141.25 ± 14.26	38.75 ± 6.20**	1296.83 ± 124.21	2.50 ± 0.05**	5.38 ± 0.09*
G5 (ACP + NAC + SMC)	396.83 ± 41.69^{++}	148.33 ± 14.03	35.83 ± 2.63*	1421.83 ± 99.72	2.62 ± 0.05*	5.28 ± 0.17**

*p < 0.05, **p < 0.01, ***p < 0.001; Comparison with control group; $^{+}$p < 0.05, $^{++}$p < 0.01, $^{+++}$p < 0.001; Comparison with only group 1 (ACP). ACP: Acetamiprid; NAC: N-acetyl cysteine; SMC: S-Methylcysteine. Each value represents the Mean ± SD.

Table 2
Effect NAC and SMC on antioxidant Parameters in the liver of rats after exposure to ACP.

	Liver Parameters				
Groups	FRAP (μM/g tissue)	MDA (nmol/g tissue)	GSH (μM/g tissue)	GST (U/mg tissue)	CAT(U/mg tissue)
Control	98.61 ± 10.18	26.81 ± 1.48	42.74 ± 3.64	313.74 ± 14.75	11.74 ± 1.16
Sham	104.20 ± 12.84^{+++}	29.82 ± 5.78+	42.41 ± 2.71+	364.26 ± 27.56^{++}	11.86 ± 1.35^{++}
G1(ACP)	42.56 ± 5.60***	47.58 ± 3.36**	29.55 ± 1.28*	531.05 ± 31.61***	20.92 ± 1.28**
G2 (NAC)	110.04 ± 6.64^{+++}	31.32 ± 2.43+	40.80 ± 2.47	389.36 ± 21.61^{++}	12.85 ± 1.79 $^{++}$
G3 (ACP + SMC)	54.13 ± 3.55**	33.06 ± 1.39	36.89 ± 3.24	429.16 ± 31.77*	14.71 ± 1.01
G4 (ACP + NAC)	66.08 ± 5.64*	36.27 ± 5.05	38.29 ± 2.26	449.02 ± 23.01**	15.93 ± 2.37
G5 (ACP + NAC + SMC)	57.46 ± 4.65**	37.56 ± 3.09	35.90 ± 1.29	455.0 ± 13.08**	14.51 ± 1.56

*p < 0.05, **p < 0.01, ***p < 0.001; Comparison with control group; $^{+}$p < 0.05, $^{++}$p < 0.01, $^{+++}$p < 0.001; Comparison with only group 1 (ACP). ACP: Acetamiprid; NAC: N-acetyl cysteine; SMC: S-Methylcysteine. Each value represents the Mean ± SD.

(P = 0.005) total antioxidant capacity of liver when compared to control group. MDA, as a biomarker of oxidative stress, was quantified in liver tissue. Statistical analysis revealed that MDA level of liver had a significant difference in the studied groups (p < 0.01). MDA level the control group was 26.81 ± 1.48 nmol/mg tissue. A more considerable increase was monitored in the MDA levels of liver tissues (47.58 ± 3.36 nmol/mg tissue, p < 0.01) of rats administered to ACP than that of the control group. Based on the data in Table 2, with slight increase groups receiving NAC and SMC, there were no significant different in ACP group treated with NAC and SMC compared to control and between them. The results of the statistical analyses including ANOVA revealed a significant difference in activity of GST in the studied groups (p < 0.001). The mean activity of GST in the control group was 313.74 ± 14.75 U/mg tissue. As shown in Table 2, a significant increase in hepatic activity of GST in group treated with ACP (531.05 ± 31.61 U/mg tissue, p < 0.001) was observed compared to the control group (313.74 ± 14.75 U/mg tissue). The groups receiving SMC and NAC as supplementation had lower activities of GST than that of the group receiving ACP. Whereas, groups receiving ACP + SMC (p < 0.05), ACP + NAC (p < 0.01) and ACP + NAC + SMC (p < 0.01) had revealed significant differences compared to the control group. The results of the statistical analyses including ANOVA revealed a significant difference in activity of CAT in the studied groups (p < 0.003). The mean activity of CAT in the control group was 11.74 ± 1.16 U/mg tissue. The results of multiple comparisons revealed a significant increase in activity of CAT in the ACP group (20.92 ± 1.28 U/mg tissue, p < 0.01) in comparison with control group (11.74 ± 1.16 U/mg tissue). The groups receiving SMC and NAC as supplementation had lower activities of CAT than that of the group receiving alone ACP (Table 3). Statistical analysis revealed that GSH level of liver had a significant difference in the studied groups (p < 0.013). GSH level the control group was 42.74 ± 3.64 μM/g tissue. A more considerable decrease was monitored in the GSH levels of liver tissues (29.55 ± 1.28 μM/g tissue, p < 0.05) of rats administered to ACP than that of the control group. Based on the data in Table 2, with slight increase GSH levels in groups receiving NAC and SMC, there were no significant different in ACP group treated with NAC and SMC compared to control and between them.

3.3. Antioxidant parameters results of brain

Statistical analysis showed that activates of GST (p < 0.001) and CAT (p < 0.001) and levels of CAT GSH (p < 0.001), MDA (p < 0.002) and FRAP (p < 0.001) had a significant difference in the studied groups. The mean activity of GST in the control group was 208.3 3 ± 18.63 U/mg tissue. The results of multiple comparisons suggested a significant increase in activity of GST in the ACP group (381.07 ± 26.43 U/mg tissue, p < 0.001) in comparison with control group (208.33 ± 18.63 U/mg tissue). The activity of GST enzyme in groups treated with ACP + SMC, ACP + NAC and ACP + NAC + SMC was significantly decreased (238.75 ± 14.72 U/mg, p = 0.007; 271. 52 ± 25.18 U/mg, p = 0.048; 265.10 ± 29.65 U/mg, p = 0.031 to 381. 07 ± 26.43 U/mg) as compared with ACP group, respectively (Table 3). The mean activity of CAT in the control group was 4.74 ± 0.38U/mg tissue. As shown in Table 3, a significant increase of CAT activity in group treated with ACP (8.25 ± 0.55 U/mg tissue, p < 0.001) was observed compared to the control group. Treatment with NAC and SMC significantly reduced all these alterations with restoration of enzyme activity (Table. 3). GSH level the control group was 45.00 ± 0.82 μM/g tissue. The results of multiple comparisons revealed a significant decrease of GSH level in the ACP group (35.24 ± 1.07 μM/g tissue, p < 0.001) in comparison with control group. Administration of NAC and SMC significantly increase levels of GSH with restoration in group 5 (p < 0.05) when compared to ACP group.

The means of MDA level in the control group was 52.90 ± 4.5 6 nmol/ mg tissue. A significant increase in the MDA level of brain was observed following exposure to ACP (91.18 ± 4.76 nmol/ mg tissue, p < 0.01) in comparison with control. Administration NAC and SMC significantly improved GSH level in experimental group. Total antioxidant level in the control group were 213.05 ± 14.27 μ M/g tissue. A significant decrease in total antioxidant level was observed following exposure to ACP (150.94 ± 6.07 μM/g tissue,

Table 3
Effect NAC and SMC on antioxidant Parameters in the brain of rats after exposure to ACP.

Groups	Brain Parameters FRAP (μM/g tissue)	MDA (nmol/g tissue)	GSH (μM/g tissue)	GST (U/mg tissue)	CAT(U/mg tissue)
Control	213.05 ± 14.27	52.90 ± 4.56	45.00 ± 0.82	208.33 ± 18.63	4.74 ± 0.38
Sham	219.23 ± 18.55^{++}	53.22 ± 4.24++	44.87 ± 1.84++	237.49 ± 22.36^{++}	5.07 ± 0.60^{++}
G1(ACP)	150.94 ± 6.07**	91.18 ± 4.76**	35.24 ± 1.07***	381.07 ± 26.43***	8.25 ± 0.55***
G2 (NAC)	209.80 ± 3.17^{++}	57.28 ± 4.90+	44.40 ± 1.61++	293.92 ± 30.05	5.21 ± 0.22 $^{++}$
G3 (ACP + SMC)	205.27 ± 4.90^{+}	67.74 ± 6.39	39.60 ± 1.99	238.75 ± 14.72^{++}	5.63 ± 0.56^{+}
G4 (ACP + NAC)	188.88 ± 9.45^{+}	68.60 ± 9.26	40.55 ± 0.65	271.52 ± 25.18^{+}	5.37 ± 0.85^{++}
G5 (ACP + NAC + SMC)	200.83 ± 7.04^{++}	70.64 ± 7.12	41.43 ± 1.49+	265.10 ± 29.65^{+}	5.61 ± 0.26^{+}

*p < 0.05, **p < 0.01, ***p < 0.001; Comparison with control group; +p < 0.05, $^{++}$p < 0.01, $^{+++}$p < 0.001; Comparison with only group 1 (ACP). ACP: Acetamiprid; NAC: N-acetyl cysteine; SMC: S-Methylcysteine. Each value represents the Mean ± SD.

p < 0.01) in comparison with control. Administration with NAC and SMC significantly improved total antioxidant level with restoration in group 3 (P = 0.007), group 4 (P = 0.047) and group 5 (P = 0.017) in compared to control group.

3.4. Histopathological findings of liver tissue

The histopathological lesions of the liver with hematoxylin-eosin staining are shown in Fig. 1 and the semiquantitative histological scoring of liver damage is classified from mild to severe (data not shown). In the control and sham groups, no histological lesions were observed and liver sections displayed normal hepatic structure of hepatocytes (Fig. 1A, B). The statistical analyses using Kruskal-Wallis Test for histopathological data indicated that there was a statistically significant difference in Congestion, hyperplasia bile duct, Sinusoidal dilatation, inflammatory cells infiltration and necrosis among the different groups (p < 0. 001). Congestion in some veins, sinusoids and increase of the number perisinusoidal cells was detected. In the group 1 (ACP), severe congestion was observed (Fig. 1I, J). Mann-Whitney post hoc tests indicated that the severity of lesions in the group 1 (ACP) was significantly higher than that of the control group (p = 0.004). In other groups, mild and moderate congestion was observed (Fig. 1C–H). Epithelial hyperplasia in the bile ducts and an increase in the number of bile ducts of the liver in the experimental group 1(ACP) was observed. According to Mann-Whitney test, mild hyperplasia was observed in ACP group that was significant in compared to control group (Fig. 1I, J). According to the classification of histopathological lesions (data not shown), there were no histopathological lesions in the hepatic structure of rats in ACP + SMC (group3), ACP + NAC (group 4) and ACP + NAC + SMC (group 5). As showed Fig. 1I, J, necrosis (pyknosis, shrinkage of a cell nucleus, with condensation of the chromatin) was moderately observed in ACP group. According to the Mann-Whitney test, these changes were significant in compared to control group. However, mild changes were observed in the rest of the experimental group (Fig. 1C–H) which showed a significant difference with the control group. Mild (Fig. 1C-H) and moderate (Fig. 1I, J) sinusoidal dilatation were observed in experimental groups. There was a significant difference between the experimental group and control group, as confirmed by Mann-Whitney test (P = 0.004). The exposure of rats to ACP induced degenerative changes in the liver organ and caused inflammatory cell infiltration by lymphocytes (Fig. 1I-J). According to the Mann-Whitney test, these changes were significant in compared to control group (P = 0.004). Coadministration of NAC with SMC and ACP showed noticeable improvement in their histological structure compared to ACP group (P = 0.005) and represented by nil to mild degree in inflammatory cellular infiltration (data not shown).

3.5. Histopathological findings of brain tissue

Histopathological alterations in the brain architecture of various groups have been illustrated in Fig. 2. In the present study, no observable histopathological changes were detected in control and sham groups (Fig. 1A, B). The statistical analyses using Kruskal-Wallis Test for histopathological data indicated that there was a significant difference in hyperemia, Gliosis and necrosis among the different groups (p < 0. 001). Histopathological findings including necrosis, hyperemia, and gliosis were observed in ACP group (Fig. 1G-J). As showed in Fig. 1G-J, Gliosis was severity detected in ACP group and Mann-Whitney post hoc tests indicated that the severity of lesions in ACP group was significantly higher than that of the control group (p = 0.004). Coadministration of NAC and SMC improve their histological structure compared to ACP group (Fig C-F). One of characteristics of necrotic cell death is red dead neurons. The neurons displayed the "red neuron or dead" appearance. Severe necrosis was observed in ACP group (Fig. 1H, J). According to the Mann-Whitney test, these changes were significant in comparison to control group (p = 0. 004). Moderate alterations were also noted in groups receiving NAC and SMC but these changes were significantly less when compared to ACP group (Fig C-F). Histopathological results showed that hyperemia was mild observed in the ACP group (Fig. 1I). According to the Mann-Whitney test, these changes were significant in compared to control group (p = 0. 003). In other groups, no obvious histopathological changes were detected.

4. Discussion

The current survey focused on the toxicity of ACP to persuade hepatic oxidative stress and whether this oxidative stress can induce brain damage, and also evaluate the extent of protective effect of either NAC or SMC. In toxicological studies, biochemical and histopathological outcomes are important criteria for the evaluation of tissue toxicity. Therefore, in this study, we used a rat model to investigate brain and hepatic damage by ACP, its possible toxic effects and also co-administration with NAC or SMC to attenuate different changes at antioxidant and biochemical and histopathological levels of liver and brain tissues.

Our study exhibit that the free radicals produced by the ACP bind covalently to protein and DNA, which cause peroxidative degradation of lipid membranes of the liver. These results suggest that toxicity of ACP play an important role in severe hepatic damage including necrosis, increased sinusoidal space, cell accumulations or severe inflammatory cells infiltration in the liver parenchyma and exit enzymes inside circulation. Aminotransferases, which are the most sensitive liver enzymes, are considered as a marker of liver tissue damage. During liver damage with an increased membrane permeability, hepatocyte cytoplasmic enzyme may leak and release from the cells into the bloodstream

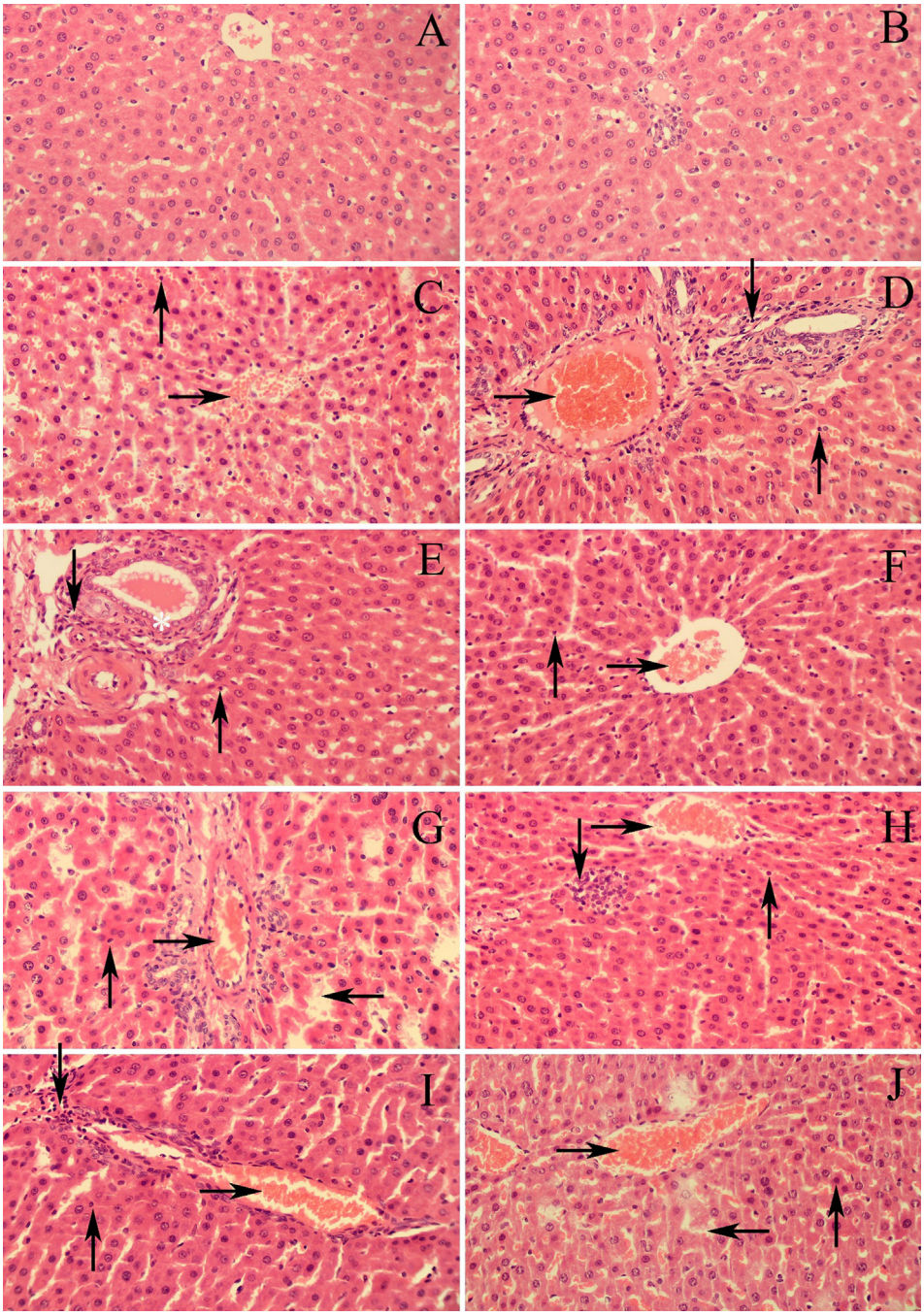

Fig. 1. The photomicrograph of histopathological changes in rat liver tissue. The control and sham rats photomicrographs (A&B; 40×, H & E) showing normal histological structure, ACP + SMC group photomicrographs (C &D, 40×, H & E), ACP + NAC group photomicrographs (E, F, 40×, H & E), ACP + SMC + NAC group photomicrographs (G, H, 40×, H & E), ACP group photomicrographs (I, J, 40×, H & E). All upward-pointing arrows indicate necrosis (pyknosis, shrinkage of a cell nucleus). All downward-pointing arrows indicate Inflammatory cell infiltration (lymphocytes). All right-pointing arrows indicate hyperemia. All left-pointing arrows indicate sinusoidal space. Hyperplasia of bile duct is marked as a white star (Figure E).

(Adaramoye et al., 2008). Therefore, the increase of these enzymes signs of damage to the liver cells, which are in agreement with our histological results and reports of previous researchers (Zhang et al., 2011; Mohany et al., 2012; Chakroun et al., 2016).

There was a significant ameliorate in the level of ALT and AST groups of receiving NAC and SMC versus ACP group, which indicates the antioxidant role of both NAC and SMC. On the other hand, the hepatoprotective effects of the treated antioxidant (NAC and SMC) have been indicated via the correlated significant decrease in the level of aminotransferases and the hepatic damage levels. Although there is substantial evidence for the impact of NAC and SMC in relation to various chemicals induced liver injury (Tahia et al., 2018), no studies pursued hepatoprotective and antioxidant activity of NAC + ACP and SMC + ACP and ACP + SMC + NAC against ACP toxicity. The present study revealed that, between the two investigated antioxidants, both have hepatoprotective (according to histopathological results) and antioxidant effect (based on biochemical analysis), in protecting the liver against ACP toxic impact. Thus, our results exhibited that NAC and SMC improved the toxic effect of ACP and resulted in fewer histopathological abnormalities

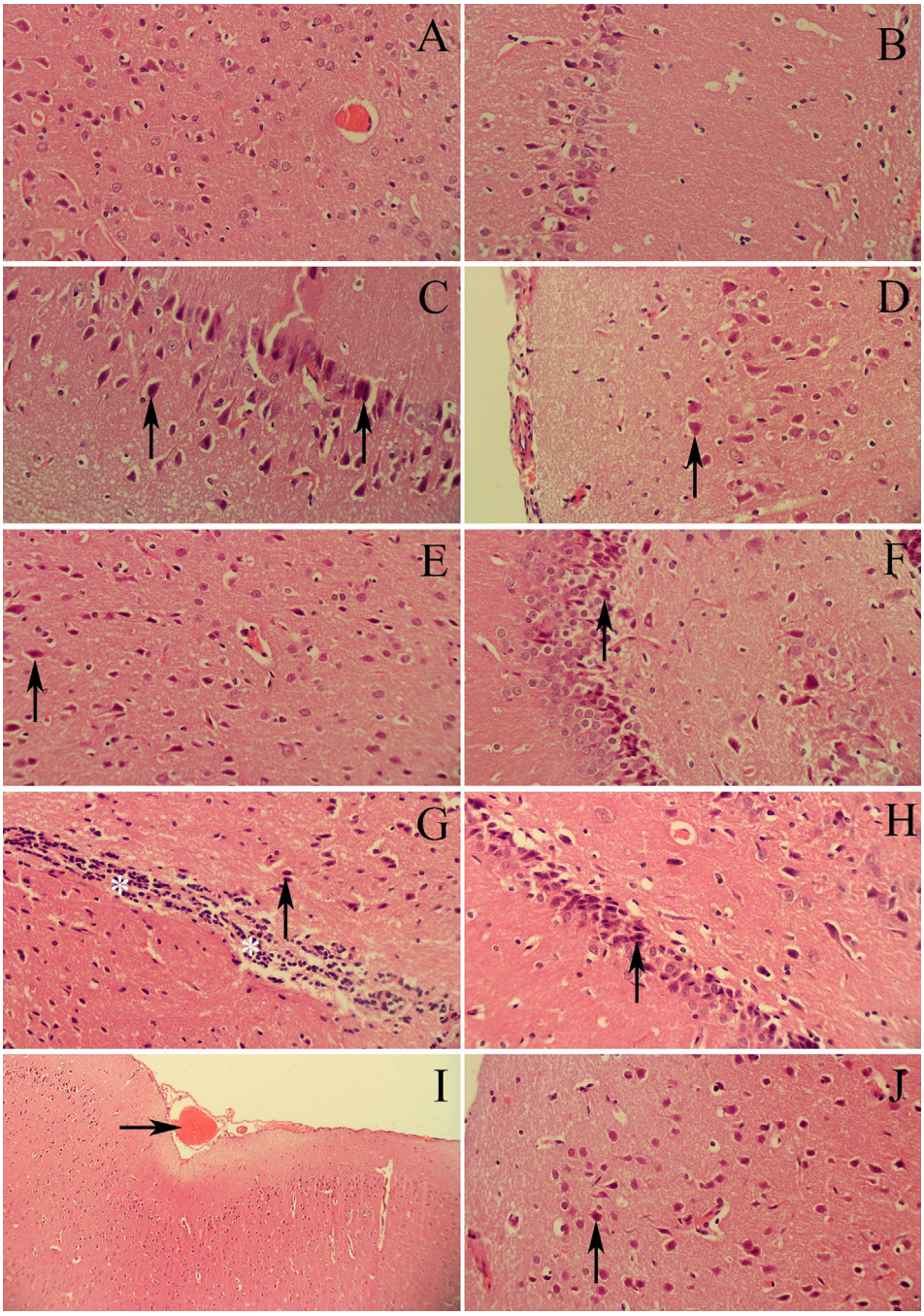

Fig. 2. The photomicrograph of histopathological changes in rat brain tissue. The control and sham rats photomicrographs (A&B; 40×, H & E) showing normal histological structure, ACP + SMC group photomicrographs (C &D, 40×, H & E), ACP + NAC group photomicrographs (E, 40×, H & E), ACP + SMC + NAC group photomicrographs (F, 40x, H & E), ACP group photomicrographs (G, H, I and J, 40×, H & E). All upward-pointing arrows indicate necrosis (the neurons displayed the "red neuron or dead" appearance). All right-pointing arrows indicate hyperemia. Gliosis is marked as a white star (Figure G).

in the liver. To check whether ACP affected liver function, also levels of serum total protein and albumin were measured. In our study, a decrease in the serum total protein and albumin was revealed in the ACP group. The reason for this decrease might be attributed to the detrimental effects of reactive oxygen species (ROS), generated by ACP in metabolic pathway, on cell structure and function. On the other hand, this was possible since ACP causes a decrease in protein metabolism and the destruction of protein synthetic machinery in the liver (Cavas et al., 2014; Chakroun et al., 2016). Our results suggest coadministration of NAC and SMC groups ameliorate the impact of ACP on liver injure which is in agreement with previous reports (Wang et al., 2012; Zhang et al., 2012). Thus, NAC and SMC as antioxidants could have protected from ACP mediated damage and prevented the leakage of protein and albumin from hepatocytes.

Oxidative stress is considered to be an important component of the mechanism of ACP toxicity; thus, the assessment of oxidative stress biomarkers is vital. In this respect, we have investigated the effect of ACP on antioxidant enzymes and lipid peroxidation. MDA, as an indicator of LPO, increases during oxidative stress indicating the level of lipid peroxidation, and its reduction indicates the activation of the antioxidant defense system (Marzban et al.,

2020). Our results display a statistically significant increase in the level of MDA liver and brain of rats exposed to ACP. Thus, oxidative stress and overproduction of ROS by ACP could be attributed to the increase of MDA level in liver and brain. The various results were achieved in the tissues of rats after exposure to ACP. Due to high unsaturated fatty acids, high susceptibility of brain to oxidative stress and consumption of 20% of body oxygen (Travacio et al., 2000), maximum lipid peroxidation was observed in brain. In addition, despite high rate of oxidative metabolism, the brain has a relatively low antioxidant defense system (Mates, 2000). Some studies indicated the increase in activities of GST and CAT concomitant with increased MDA level in liver and testis of rats (Zhang et al., 2011; Chakroun et al., 2016). These findings are in agreement with our results.

On the contrary, other studies showed a decrease in activity CAT and increase lipid peroxidation in the rat by ACP and imidacloprid (Kapoor et al., 2010; Devan et al., 2015). Therefore, contradictory results can be due to the type of chemical substance, route of administration, dose, and time of exposure. Coadministration of rats with NAC and SMC groups led to the improvement of MDA level, by ameliorating the oxidative stress in hepatocytes.

Antioxidant, neuroprotective, and anti-inflammatory effects of S-allyl cysteine of garlic were observed in rat hippocampus (Sadeghi et al., 2013; Colin-Gonzalez et al., 2015) and the protective effects of garlic compounds are attributed to its antioxidant properties (Aguilera et al., 2010). Previous studies revealed that thiol-containing compounds such as the S-allyl cysteine and S-methyl cysteine of garlic prevent lipid peroxidation and protect the nervous system of the mouse brain by inhibiting the lipid peroxidation (Numagami and Ohnishi, 2001; Rojas et al., 2011; Manoj Kumar et al., 2017).

Total antioxidant capacity (TAC), as a biomarker, can evaluate the antioxidant response against the free radicals produced by a chemical substance (Valko et al., 2007). Therefore, the keeping of sufficient antioxidant levels is necessary to prevent disease conditions. According to the results obtained in our study, the decrease of total antioxidant capacity can be attributed to the increased lipid peroxidation which in line with our observations. Therefore, the production of ROS by ACP and increasing oxidative stress appear to be related to the reduction of antioxidant capacity.

Catalase is one of the most important antioxidant defense enzymes against prooxidants that catalyzes the decomposing of hydrogen peroxide to H_2O and O_2 at high catalytic rates. The activity of this enzyme may fluctuate during oxidative stress. Therefore, measuring the activity of the catalase can be an indicator for the evaluation of many diseases. In the present study, the activity of CAT increased significantly in the brain by ACP and this is in agreement with previous findings (Mansour et al., 2009; Colović et al., 2015). The increase of catalase activity is probably due to antioxidant defense mechanisms against prooxidants. In fact, the antioxidant defense system, by increasing catalase, prevents increase H_2O_2 and tissue damage caused by ACP. In fact, short-term exposure to ACP may produce ROS, which the organism defense system attempts to modulate the toxic effect by increasing catalase activity, but when prolonged ACP injections, the overproduction of ROS levels and decrease of catalase activity, the reason for the weakening of antioxidant defense system to remove ROS. In our study, the administration of the two tested antioxidants resulted in attenuation of oxidative stress, which may be due to the ability of this antioxidant to directly remove free radicals, and consequently improvement of catalase activity in brain and liver, especially with the NAC compound.

The brain is the most susceptible organ to oxidative injure due to its biochemical and physiological properties. To further confirm the brain damage induced by ACP, histopathological alterations were investigated. Our study revealed Gliosis, Hyperemia, and Necrosis of brain tissue by ACP. These changes were also observed with Organophosphorus compounds such as dimethoate on antioxidant status of liver and brain in male Wistar rats (Sharma et al., 2005; Singh et al., 2013). Exposure to ACP causes severe degenerative changes in brain tissue and these changes were improved with SMC and NAC treatment. Neuroprotective effects of N-acetylcysteine observed in a brain injury that outcomes this study result in ameliorating inflammatory response and biochemical alteration in the rat model (Chen et al., 2008; Hicdonmez et al., 2006). NAC as a neuroprotective agent in preclinical models of central and peripheral nervous injury has been shown to have antioxidant and neurovascular-protective effects by repairing traumatic brain injury (Yi et al., 2006; Chen et al., 2008; Eakin et al., 2014).

The GST enzyme and GSH were other parameters in the antioxidant defense systems that were studied in this research. Our results demonstrate a statistically significant decrease in GSH level and increase of GST activity in the liver of rats exposed to ACP. GSH, a tripeptide containing L-cysteine, L-glutamic acid and glycine, can directly remove free radicals or act as a substrate for glutathione S-transferase during the detoxification of hydrogen peroxide. Group (SH) of cysteine in glutathione plays an important role in intracellular antioxidant. Thus, NAC can act as a precursor for the synthesis of glutathione and support the potential role of GSH in the blood-brain barrier. Studies revealed that despite its poor penetration into the CNS, NAC can significantly elevate GSH levels in the brain after oxidative stress (Farr et al., 2003; Lanté et al., 2008) and GSH deficiency (Das Neves Duarte et al., 2012). The protective effect of NAC can be attributed to its sulfhydryl group or probably a result of direct scavenging of toxic oxygen species. These observations are consistent with another study and suggest that NAC prevents oxidation of GSH by its own oxidant scavenging capacity, rather than improving the synthesis of GSH. The increase of GST activity in the liver tissue by the ACP group designates to its role in the detoxification of ACP. Our results are in agreement with previous researchers after exposure to other insecticides (Sivapiriya et al., 2006; Yu et al., 2008; Heikal et al., 2012). In contrast, other studies indicated a reduction in the activities of antioxidant enzymes that attributed to the inability of the organism to counteract the overproduction of ROS (Mahboob and Siddiqui, 2001). On the other hand, co-administration of both NAC and SMC revealed an effective effect against oxidative stress, lipid peroxidation and attenuating hepatic GST activity. These findings were in agreement with the universal opinion that oxidation of GSH a parameter of oxidative stress.

5. Conclusions

NAC and SMC have a broad spectrum of possible and potential applications to facilitate recovery after liver and brain injury. Our results support an antioxidant and protective role of NAC and SMC to attenuate the hepatotoxicity and brain damage in the rat after short term exposure to ACP. NAC showed an approximate similar effect with SMC in neutralizing the hepatic oxidative stress induced by ACP. Thus, our data indicate between the two investigated antioxidants, both have a hepatoprotective and antioxidant effect in protective tissues against ACP toxicity. One of the major limitations of NAC is low bioavailability relative to oxidative stress-related diseases. To overcome this limitation, the synthesize of amide derivative of NAC (more lipophilicity) is suggested to improve membrane permeability and antioxidant properties. However, the exact mechanism of NAC and SMC as an antioxidant is not yet obvious. So, further studies should be performed to understand the precise protective mechanism of NAC and SMC against ACP-induced oxidative stress.

6. Compliance with Ethical Standards

This study was carried out in agreement with the Guide of Care and was approved by the ethics committee on animal experimentation of the University of Mazandaran (UMZ. REC.396005).

7. Fun**ding Information

This work is supported by the University of Mazandaran, Babolsar, Iran.

8. Consent for Publication

All the authors agreed for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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