

# Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats

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**Abstract** Acetamiprid is one of the most widely used neonicotinoids. This study investigates toxic effects of repeated oral administration of three doses of acetamiprid (1/20, 1/10, and 1/5 of LD<sub>50</sub>) during 60 days. For this, male Wistar rats were divided into four different groups. Hematological, biochemical, and toxicopathic effects of acetamiprid were evaluated. According to the results, a significant decrease in the body weight gain at the highest dose 1/5 of LD<sub>50</sub> of acetamiprid was noticed. An increase in the relative liver weight was also observed at this dose level. The hematological constituents were affected. A significant decrease in RBC, HGB, and HCT in rats treated with higher doses of acetamiprid (1/10 and 1/5 of LD<sub>50</sub>) was noted. However, a significant increase in WBC and PLT were observed at the same doses. Furthermore, acetamiprid induced liver toxicity measured by the increased activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALPs), and lactate dehydrogenase (LDH) which may be due to the loss of hepatic membrane architecture and hepatocellular damage. In addition, exposure to acetamiprid resulted in a significant decrease in the levels of superoxide dismutase and catalase activities ( $p \leq 0.01$ ) with concomitant increase

in lipid peroxidation in rat liver. These findings highlight the subchronic hepatotoxicity of acetamiprid.

**Keywords** Acetamiprid · Hematological and biochemical parameters · Hepatotoxicity · Oxidative stress

## Introduction

It is obvious that our environments as well as our health are constantly threatened by various pollutants such as xenobiotics. Pesticides are one of the most common pollutant groups in the world, and they have a major drawback such as toxicity (Speck-Planche et al. 2012). The continuous use of pesticide imposes hazardous effect on the physiological function of various body systems (Singh et al. 2012). Thus, long-term exposure can be harmful to human life and can disturb the functioning of different organs in the body (Hamadache et al. 2016). Neonicotinoids are a relatively new class of pesticides used as insecticides (Cavas et al. 2014). As they are widely applied throughout the world, they are creating a real public concern (Pisa et al. 2015) which has been shown in a wide range of studies devoted by research groups in many countries, as may be confirmed by the latest conclusions of the Worldwide Integrated Assessment group (Van der Sluijs et al. 2015). Acetamiprid (ACMP), (E)-N-[(6-chloro-3-pyridyl)methyl]-N-cyano-N-methylacetamide, is a member of the newly developed neonicotinoid group of insecticides commonly used against a large variety of insect pests (Sanyal et al. 2008). Moreover, ACMP is frequently detected in agricultural products owing to its widespread and extensive use (Akiyama et al. 2002) and because of its potential toxicity to humans (Pramanik et al. 2006; Sanyal et al. 2008). It acts as a selective agonist for the nicotinic acetylcholine receptors in insects (Shimomura et al. 2006). Despite the interaction

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between effects of exposure to various toxic chemicals in the environment and the survival of human being, toxicological studies of ACMP are limited and cases of ACMP poisoning are still rare. Few studies have shown the acute or chronic toxic effects of ACMP. Nevertheless, people suffered from headaches, dizziness, nausea, vomiting, and other symptoms after the inhalation of ACMP (Todani et al. 2008).

The imbalance between the production of oxygen free radicals (OFRs) and antioxidant defenses in the body is called oxidative stress which has important health implications (Mansour and Mossa 2010). Liver has been considered as the most important organ targeted by the toxic effects of xenobiotic such as pesticide. Pesticide exposure has been linked to oxidative stress (Merhi et al. 2010). Available reports indicate that insecticides alter the enzyme activities associated with antioxidant defense mechanisms (Akhgari et al. 2003; Ranjbar et al. 2002; Shadnia et al. 2005). Doubtless to say that there are some antecedent reports performed in mammals about the susceptibility to oxidative damage in response to oxidative stress induced by ACMP. Therefore, given the widespread use of ACMP and the lack of information concerning the subchronic studies of commercial ACMP preparation, this paper highlights the investigation of the adverse effects of subchronic exposure to ACMP which caused changes in hemato-biochemical parameters, oxidant/antioxidant status, and histopathological alterations of rat liver exposed to ACMP.

All in all, the findings have provided new insights on the ACMP toxicological impacts which may shed light on their possible hazards to the environment and human health.

## Materials and methods

### Chemical product

Commercial product of ACMP (Mospilan®20 SL, consisting of 200 g/L ACMP as active ingredient) is manufactured by ArystaLifeScience, Nippon SodaCo. Ltd., Japan.

### Animals

Adult male Wistar rats aged of 4 months were obtained from SIPHAT (Pharmaceutical Industrial Society of Tunisia, Ben Arous, Tunisia). Animals were divided into four groups of six animals each, one control group, and three ACMP-treated groups. They were acclimatized a week before the onset of experiment and were maintained under controlled conditions of temperature ( $25 \pm 2$  °C) and humidity (55 %) with a 12-h light/dark cycle. They were provided with standard commercial pellet diet from Sico Sfax (Tunisia) and drinking water ad libitum. Animals were maintained during

the experimental period in accordance with the guidelines for animal care of the “Faculty of Medicine of Monastir,” Tunisia.

### Experimental design

The intragastric ACMP doses were selected according to published data (Yamada 1997) which indicate the acute oral LD<sub>50</sub> value of ACMP to be 217 mg/kg in male rats. The body weight of controls as well as treated rats were taken weekly throughout the experiment and then on the day of sacrifice.

For the preparation, dosage of each solution administrated was daily freshly prepared and adjusted weekly for body weight changes.

#### 1. Treated groups:

ACMP was dissolved in distilled water of pharmaceutical quality, and we have proceeded to give intragastrically 5 ml/kg of body weight of insecticide every day for 60 days. The test concentrations were calculated depending on the percentage of active ingredients of commercial formulation of ACMP.

Group 1: 1.6 ml of Mospilan 20 SL was dissolved in 28.4 ml of distilled water and administered at a dose of 1/20 of LD<sub>50</sub> (10.85 mg/kg).

Group 2: 3.2 ml of Mospilan 20 SL was dissolved in 26.8 ml of distilled water and administered at a dose of 1/10 of LD<sub>50</sub> (21.7 mg/kg).

Group 3: 6.5 ml of Mospilan 20 SL was dissolved in 23.5 ml of distilled water and administered at a dose of 1/5 of LD<sub>50</sub> (43.4 mg/kg).

#### 2. Control group: received orally an equivalent volume of distilled water as previously described for treated groups.

### Blood and liver tissue sample

At the end of experiment period, rats were fasted overnight, and 24 h after the last administration of ACMP, they were subsequently anesthetized. Blood sample was collected by cardiac puncture from each rat for hematological studies while plasma was extracted by centrifugation of the whole blood at 3000×g for 15 min at 4 °C, which was then conserved at -20 °C for further biochemical analysis, such as total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALPs), and lactate dehydrogenase (LDH).

The livers of these animals were extracted quickly, perfused immediately with ice-cold physiological saline (0.9 % w/v), blotted dry, and weighed. The liver tissues were fixed in 10 % buffered-neutral formalin and embedded in paraffin.

About 1 g of the liver was minced and homogenized (10 % w/v) with a Potter-Elvehjem type homogenizer in 5 ml of ice-cold phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000×g for 20 min at 4 °C. Supernatants were collected and stored at –80 °C until use for evaluation of lipid peroxidation and antioxidant enzyme activities.

### Investigations of histopathological alterations

After routine histological laboratory procedures, sections of 5 µm were cut and stained with hematoxylin and eosin (H&E) for histopathology. The sections were viewed and photographed by using a Leica light microscope (Leica DM750), provided with a camera (Leica ICC50). Six microscopy stained slides per animal were examined for signs of histopathological features, such as hypertrophy of central vein, dilated portal triad, lymphoid infiltration, dilatation of sinusoids, vacuolization, and necrosis. Each liver slide was examined, and the severity of the changes observed were scored using a scale of none (–), mild (+), moderate (++) and severe (+++) damage.

### Biochemical evaluation

Blood samples were collected in heparinized centrifuge tube and centrifuged. The plasma enzyme levels including total proteins, AST, ALT, ALP, and LDH activities were determined spectrophotometrically from plasma samples using commercial reagent kits from Roche Diagnostics GmbH (Mannheim, Germany).

### Hematological parameters

Blood was collected in EDTA-treated tubes. Red blood cell count (RBC, 10<sup>12</sup>/L), hemoglobin concentration (HGB, g/L), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentrations (MCHC, g/dL), platelet count (PLT, 10<sup>9</sup>/L), mean platelet volume (MPV, fL), and white blood cell count (WBC, 10<sup>9</sup>/L) were quantified in an automatic hematological assay analyzer (nine-parameter system) (Medonic Cell Analyzer-Vet CA530, Boule Medical, Stockholm, Sweden) (Martensson et al. 2010) at “Fattouma Bourguiba University Hospital” in Monastir (Laboratory of Hematology).

### Estimation of lipid peroxidation

Concentration of lipid peroxidation in liver tissue homogenate of all the experimental animals was assessed by determining the rate of the production of thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust (1978). A volume of 125 ml of supernatant was mixed with

50 ml of Tris-buffered saline (TBS, pH 7.4) and 125 ml of 20 % trichloroacetic acid containing 1 % butylated hydroxytoluene (BHT), and centrifuged (1000×g, 10 min, 4 °C). Then, 200 ml of supernatant were mixed with 40 ml of HCl (0.6 M) and 160 ml of thiobarbituric acid (120 mM) dissolved in Tris, and the mixture was heated at 80 °C for 10 min. The absorbance was read spectrophotometrically at 530 nm and was proportional to the amount of TBARS formed. Values were expressed as nmol malondialdehyde (MDA) equivalents per mg protein.

### Evaluation of antioxidant enzymes

#### *Catalase activity*

Hepatic catalase activity was measured according to Clairbone (1985). Briefly, 20 µl of the homogenate was added in the quartz cuvette containing 780 µl phosphate buffer and 200 µl of H<sub>2</sub>O<sub>2</sub> 0.5 M. The enzyme activity was measured by determining the maximum absorbance at 240 nm using the molar extinction coefficient of 0.04/mM/cm. One unit of activity is equal to the µmol of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein.

#### *Assay of SOD*

The assay for superoxide dismutase activity (SOD) activity in the liver was made according to the method of Suttle (Zhang et al. 2010) using Ransod kit (Randox Labs, Crumlin, UK). This method is based on the formation of red formazan from the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride and superoxide radical (produced in the incubation medium from xanthine oxidase reaction), which is assayed in a Thermo Electron Multiskan Ascent photometer (Vantaa, Finland), and the wavelength was monitored at 505 nm. The inhibition of the produced chromogen is proportional to the activity of the SOD present in the sample. A 50 % inhibition is defined as 1 unit of SOD, and specific activity is expressed as units per milligram of protein.

#### *Assay of GPx*

Glutathione peroxidase activity (GPx) activity in the liver was performed according to the method of Flohe and Gunzler (1984). The activity was expressed as µmol of GSH oxidized/min/mg of protein, at 25 °C.

#### *Statistical analysis*

Data were expressed as means ± standard deviation (SD) based on the indicated number in the experiment ( $n = 6$ ). They were analyzed using the Statistical Package for Social Sciences (SPSS, version 18). Statistical significance was determined using one-way analysis of variance (ANOVA)

followed by Tukey's post hoc comparisons. Differences were considered statistically significant when  $p$  value was  $\leq 0.05$ .

## Results

### Body weight gain and relative organ weight

No deaths were reported after oral administration of ACMP. No physical signs of toxicity or adverse effects to treatment were observed. At the end of experiment, we observed a decrease of body weight of treated rats (Fig. 1). This decrease was significant in rats treated with the highest dose of ACMP in comparison with the control group ( $p \leq 0.01$ ).

We observed a significant increase in relative liver weight of treated rats with the highest dose of ACMP in comparison with control and other treated groups ( $p \leq 0.01$ ) (Fig. 2).

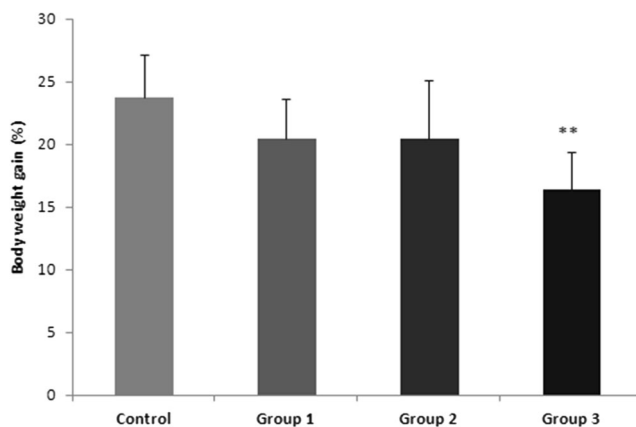
### Effect of treatment on hematological parameters

The toxicology results of ACMP on hematological parameters are shown in Table 1. A statistically significant increase in WBC and PLT was observed in rats treated with higher doses ( $p \leq 0.01$ ). Similarly, a significant increase in MCV was observed only in the group treated with the highest dose ( $p \leq 0.01$ ) whereas a significant decrease in RBC, HGB, and HCT in treated groups with higher doses was observed ( $p \leq 0.05$  or  $0.01$ ).

The values of hematological parameters such as MCH, MCHC, and MPV were not significantly different in treated groups versus control group.

### Evaluation of modifications on biochemical parameters

The toxicological results of ACMP on biochemical parameters are shown in Table 2. The activity of plasma enzymes

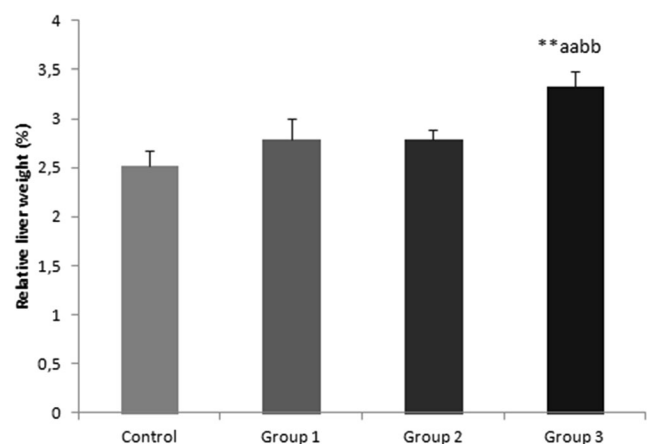


**Fig. 1** Effect of ACMP on body weight gain in the treated and control groups. Values represent means  $\pm$  SD. \*\*Significantly different from the control at 1 % confidence level

(AST, ALT, and LDH) was significantly increased after 60 days of exposure to higher doses when compared to the control group ( $p \leq 0.05$  or  $0.01$ ). On the other hand, the activity of ALP was significantly increased for all treated groups compared to control group ( $p \leq 0.01$ ) while the comparative analysis of total protein was significantly decreased in rats exposed to higher doses ( $p \leq 0.01$ ). The results revealed that ACMP changed plasma biomarkers in a dose-dependent manner.

### Histological analysis of liver tissue

Under light microscope, histopathological analysis of liver tissue sections of rats exposed to ACMP showed abnormalities as compared to the control group. The histological examination of the control liver tissue exhibited normal structure of hepatocytes, large, hexagonal in shape, which were arranged in anatomizing cords, radiating from central vein to the normal portal triad, separated from each other by blood sinusoids. The nuclei of hepatocytes had a normal vesicular structure, and the cytoplasm appeared normal and uniform (Fig. 3a, d). Liver of rats exposed to the lowest dose did not induce marked changes in its histological structure. Mild dilated central vein and portal triad were observed (Table 3). However, alterations in rat liver were accentuated after the administration of 21.7 mg/kg bw (group 2). Indeed, we observed moderate alterations such as central vein hypertrophy associated with sinusoid dilatation (Fig. 3b) and a dilated portal triad was noted (Fig. 3e). We also observed some areas with leukocyte infiltrates (Fig. 3g). These alterations were increased in hepatic parenchyma of rats exposed to the highest dose (43.4 mg/kg bw). There was severe dilated central vein (Fig. 3c), severe dilated portal triad (Fig. 3f), and inflammatory leukocyte infiltrations (Fig. 3h). In addition, we detected a severe dilatation of sinusoid capillaries (Fig. 3i) associated with extensive cytoplasmic vacuolization



**Fig. 2** Effect of ACMP on relative liver weight in the treated and control groups. Values represent means  $\pm$  SD. \*\*Significantly different from the control at 1 % confidence level; <sup>aa</sup>significantly different from the group 1 at 1 %; <sup>bb</sup>significantly different from the group 2 at 1 %

**Table 1** Effect on hematological parameters of male rats after 60 days of the intragastric administration of ACMP

Parameters	ACMP (mg/kg bw)			
	Control	Group 1	Group 2	Group 3
Parameters	0	10.85	21.7	43.4
WBC (10 <sup>9</sup> /L)	9.33 ± 0.92	9.88 ± 1.28	20.75 ± 0.67**	21.93 ± 1.69**
RBC (10 <sup>12</sup> /L)	7.56 ± 0.37	7.14 ± 0.43	6.50 ± 0.38**	6.13 ± 0.82**
HGB (g/L)	12.80 ± 0.22	11.70 ± 1.06	11.41 ± 0.68*	11.17 ± 0.79**
MCV (fL)	48.70 ± 0.87	49.16 ± 2.18	50.10 ± 2.08	56.36 ± 1.27**
MCH (pg)	18.05 ± 0.36	17.71 ± 0.58	17.50 ± 0.36	17.40 ± 1.30
MCHC (g/L)	35.72 ± 1.16	35.21 ± 0.69	35.12 ± 0.96	36.78 ± 1.46
PLT (10 <sup>9</sup> /L)	399 ± 28.54	417.66 ± 52.30	520.16 ± 37.74**	555.83 ± 41.74**
HCT (%)	37.44 ± 1.36	36.20 ± 1.05	33.60 ± 1.25**	20.95 ± 1.52**
MPV (fL)	7.72 ± 0.43	8.07 ± 0.75	8.16 ± 0.31	8.36 ± 0.71

All values are expressed as mean ± SD

\*Significantly different from control at 5 %; \*\*significantly different from control at 1 %

(Fig. 3j). Also, some necrotic hepatocytes were observed (Fig. 3k). A few hepatocytes were characterized by the appearance of small darkly stained nuclei (chromatin condensation as a result of apoptosis), and we noted the formation of edema and the dislocation of the wall of the central vein (Fig. 3l). These observations confirmed that ACMP has potential to induce hepatotoxicity in rats in a dose-dependent manner. All of these histopathological changes were graded and are summarized in Table 3.

**Effects on MDA level, SOD, GPx, and catalase activities**

In this study, MDA was used as a marker of lipid peroxidation (LPO) of liver in treated rats. As shown in Table 4 and after a subchronic exposure to ACMP, a significant increase in hepatic level of MDA in animals treated with higher doses was observed compared to the control group ( $p \leq 0.05$  or  $0.01$ ). Also, treatment with the highest dose led to a significant increase in MDA compared to the lowest dose ( $p \leq 0.01$ ).

Based on the data in Table 4, there were no significant changes in GPx activity in all treated groups compared to control and between them while SOD and CAT activities were significantly decreased in different treated groups compared to control group ( $p \leq 0.01$ ) and other ACMP groups. The activities of SOD and CAT showed a statistically highly significant dose-dependent decrease in treatment with higher doses compared to the group treated with the lowest dose ( $p \leq 0.01$ ). The present results illustrate that ACMP exposure causes statistically significant changes in oxidative stress biomarkers (LPO, SOD, and CAT) in the liver homogenate in a dose-dependent manner.

**Discussion**

The neonicotinoids have unique physical and toxicological properties as compared to earlier classes of organic insecticides. The mammalian toxicity of neonicotinoids is

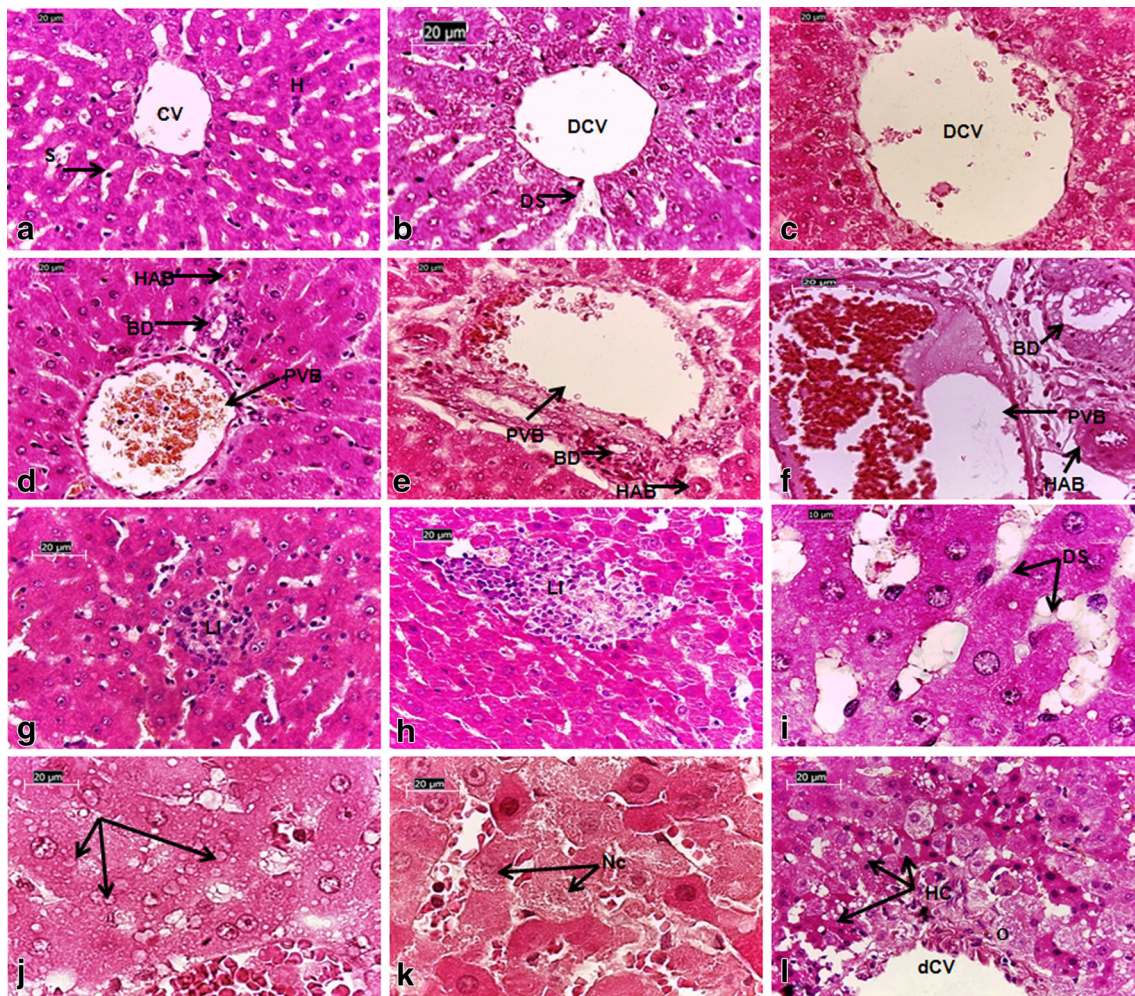
**Table 2** Enzyme activities in the plasma of control and rats treated with ACMP

Enzyme	ACMP (mg/kg bw)			
	Control	Group 1	Group 2	Group 3
Enzyme	0	10.85	21.7	43.4
AST (UI/L)	59.87 ± 1.43	67.62 ± 10.65	88.6 ± 13.96***	105.15 ± 14.85**.#
ALT (UI/L)	21.25 ± 2.12	26.42 ± 3.66	30.38 ± 8.10*	56.9 ± 6.17**.#,###
ALP (UI/L)	52.42 ± 1.83	90 ± 7.07**	130 ± 4.04**.#	166 ± 11.20**.#,###
LDH (UI/L)	260.16 ± 29.53	283.16 ± 44.55	396 ± 49.09***	554.33 ± 60.44**.#
Total protein (g/L)	63.17 ± 3.09	60.5 ± 1.84	58 ± 2.51**	57.86 ± 0.79**

Values represent means ± SD

\*Significantly different from the control at 5 % confidence level; \*\*significantly different from the control at 1 % confidence level; \*\*\*significantly different from the group 1 at 5 %; # significantly different from the group 1 at 1 %; ### significantly different from the group 2 at 1 %





**Fig. 3** Light microscopy of histological sections of hematoxylin-eosin (H&E)-stained liver from control and treated groups. Histological sections of control animals showed normal liver parenchyma and a normal portal triad (**a**, **d**) ( $\times 400$ ). Liver sections of group 2 (rats treated with 21.7 mg/kg ACMP) demonstrated hypertrophied central vein (**b**) ( $\times 400$ ), dilated portal triad (**e**) ( $\times 400$ ), and moderated leukocyte infiltrates (**g**) ( $\times 400$ ). Liver sections of group 3 (rats treated with 43.4 mg/kg) exhibited severe dilated central vein (**c**) ( $\times 400$ ), and severe dilated portal

triad (**f**) ( $\times 400$ ). Liver sections of rats treated with 43.4 mg/kg (group 3) exhibiting severe alterations in the parenchyma (**h–l**) ( $\times 400$ ,  $\times 1000$ ,  $\times 1000$ ,  $\times 1000$ , and  $\times 400$ , respectively). *CV* central vein, *S* sinusoids, *H* hepatocyte, *V* vacuoles, *Nc* necrotic cell, *HC* hyperchromatic nuclei, *O* edema, *DCV* dilated central vein, *DS* dilated sinusoid, portal triad, *PVB* portal vein branch, *HAB* hepatic artery branch, *BD* bile duct, *dCV* dislocated central vein, *LI* inflammatory leukocyte infiltrations

considered to be centrally mediated because the symptoms of poisoning are similar to those of nicotine (Tomizawa and Casida 2005). However, information on its toxicity to mammalian is limited. Thus, in the current study, we used a commercial product of ACMP in order to evaluate its possible toxic effects on rats due to intragastric administration for 60 days.

In the present study, a decrease in body weight gain was observed. That decrease was significant only in group treated with the highest dose of ACMP in comparison with the control group. In the same way, a recent study (Devan et al. 2015) showed a significant reduction in body weight gain in both sexes at high dose of ACMP.

Similarly, Zhang et al. (2010) and Singh et al. (2012) reported a reduction in body weight of mice treated

with ACMP for 28 and 35 days, respectively. Thus, the reduction of body weight gain noticed after ACMP exposure could be predominantly due to a food intake disturbance.

However, an increase in relative liver weight was observed. That increase was significant only in rats treated with the highest dose. The same finding was proved by Bhardwaj et al. (2010) after administration of 20 mg/kg/day of imidacloprid (another neonicotinoid). The increase in the relative liver weight in animals treated with the highest dose might be due to the edema observed in the liver tissue sections. In fact, an increase in the absolute liver weight (Sharma et al. 2005) and relative liver weight (Undeger et al. 2000) can be caused by some pesticides in experimental animals.

The present study reveals statistically significant changes of some hematological parameters of rats treated

**Table 3** Histopathological alterations in the liver of rats, exposed to different concentrations of ACMP

ACMP (mg/kg bw)				
	Control	Group 1	Group 2	Group 3
Histopathological alterations	0	10.85	21.7	43.4
Hypertrophied central vein	–	+	++	+++
Dilated sinusoid	–	–	++	+++
Dilated portal triad	–	+	++	+++
Lymphoid infiltration	–	–	++	+++
Vacuolization	–	–	++	+++
Necrosis	–	–	–	++
Hyperchromatic nuclei	–	–	–	+
Edema	–	–	–	++
Dislocation of central vein	–	–	–	+

– none, + mild, ++ moderate, +++ severe

with the highest dose of ACMP. The hematological findings showed a significant decrease in RBC, HGB, and HCT levels relative to the control group as found by Singh et al. (2012) in ACMP-treated mice. Moreover, we also found an increase in MCV level in the group treated with the highest dose, which might be a symptom of macrocytic anemia (Grissa et al. 2015). Our results indicate an increment in WBC and PLT counts. The increase in PLT count might indicate a possible effect of ACMP on blood coagulation and fibrinolysis systems. Whereas, the observed leukocytosis may be due to increased leukocyte mobilization and can be directly proportional to the severity of the causative stress condition (Celik et al. 2009). Therefore, the reason for this increase could be explained by the immune system activation and to the presence of inflammation in liver tissues (Yousef et al. 2003). This possibility is in concordance with our histopathological analysis after treatment with higher doses of ACMP which caused lymphoid infiltration. In

fact, the lymphocytic infiltrates observed is an indicator of cell irritability and inflammation. Other visual consequences were observed such as cytoplasmic vacuolation, dilatation of sinusoid capillary, central vein and portal triad, pycnotic nuclei, necrosis of the hepatocyte, edema, and dislocation of the wall of the central vein. This histopathological analysis revealed cellular injury in rat liver, and their severity seems to be dose dependent. These observations were in accordance with those obtained by other previously published findings in liver (Bhardwaj et al. 2010), like mild focal necrosis and hepatocellular damage after subchronic and acute imidaclopride exposure. Also, dilatation of central vein and blood sinusoids, pycnotic nuclei, and leukocyte infiltration were observed in hepatic tissue of female rats exposed to imidacloprid at 45 mg/kg bw for 4 weeks (Toor et al. 2013).

The results of light microscopic analyses support the biochemical studies. Aminotransferases are marker enzymes for liver function and integrity (Adaramoye et al. 2008). In this study, significant increases in plasma levels of AST and ALT were observed in higher doses of ACMP-treated rats. A significant increase of ALP was also observed in blood plasma of highest dose-treated group. Our results are in accordance with those reported by Zhang et al. (2011) which have found that ACMP increased the levels of AST, ALT, and ALP. Also, Mohany et al. (2011) have reported similar findings after repeated oral administration of imidacloprid over 4 weeks in albino rats.

In our study, the elevation of blood enzymes activity may be caused by the loss of hepatic membrane architecture and hepatocellular damage observed in higher dose-treated animals which contribute to the leakage of the intracellular enzymes into the blood. It has been demonstrated that cell damage exhibited good correlation with the enzyme leakage (Awad et al. 1998).

Lactate dehydrogenase is clinically the most important of several dehydrogenases occurring in blood serum (Tomaszewska et al. 2015). The current study indicated a

**Table 4** Effect on oxidant and antioxidant enzyme activities in the liver of rats after 60 days of intragastric administration of ACMP

ACMP (mg/kg bw)				
	Control	Group 1	Group 2	Group 3
Parameters	0	10.85	21.7	43.4
MDA (nmol MDA/mg protein)	0.33 ± 0.13	0.40 ± 0.14	0.66 ± 0.20*	0.92 ± 0.27***
GPx (µmol GSH oxidized/min/mg protein)	2.32 ± 0.19	2.36 ± 0.55	2.54 ± 0.07	2.63 ± 0.58
SOD (unit/mg protein)	33.21 ± 2.16	27.23 ± 1.81**	20.74 ± 1.10***	19.71 ± 1.75***
CAT (µmol/min/mg protein)	638.39 ± 7.48	415.09 ± 6.59**	290.21 ± 41.13***	209.94 ± 30.64***

Values represent means ± SD

\*Significantly different from the control at 5 % confidence level; \*\*significantly different from the control at 1 % confidence level; \*\*\*significantly different from the group 1 at 1 %; # significantly different from the group 2 at 1 %



significant elevated level of LDH in rats exposed to the higher doses of ACMP. Also, the increase in plasma LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme into the blood stream (Wang and Zhai 1988).

Total protein is done as a routine test to evaluate the toxicological nature of various chemicals (Mansour and Mossa 2010). In the present study, ACMP exposure reduced significantly the total protein levels at the higher doses. The reason of this decrease in total protein could be due to the damaging effects of reactive oxygen species (ROS), generated by ACMP exposure on the proteins by oxidation in the liver. Obviously, although neonicotinoid pesticides have different modes of action, it is known to produce oxidative stress by inhibiting antioxidant enzymes as well as by inducing generation of ROS (Cavas et al. 2012; Zhang et al. 2011, 2012).

In this respect, we are focused on the study of the effect of ACMP on antioxidant enzymes and lipid peroxidation. Antioxidant can inhibit free radical formation. Our result shows a statistically significant decrease in the antioxidant enzymes activities (SOD and CAT) with concomitant increase in MDA level, as indicator of LPO, in liver of rats exposed to the higher doses. Similar results were obtained in the testis of mice after subacute exposure to ACMP (Zhang et al. 2011). Furthermore, Devan et al. (2015) showed that ACMP decreased SOD and CAT and increased lipid peroxydation in the liver of rats. Kapoor et al. (2010) investigated the effects of imidacloprid on SOD and CAT activities and lipid peroxidation following subchronic dermal exposure in female rats and found out identical results. Also, Ince et al. (2013) reported that oral administration of 15 mg/kg/day of imidacloprid for 28 days produced a significant elevation in MDA level and decreased SOD and catalase activities in mice. However, other findings reported that ACMP increased the level of SOD and CAT enzymes in bacteria (Yao et al. 2006) and in plants (Ford et al. 2011). In fact, increased SOD and CAT activities observed in these studies could be caused by an elevated level of ROS induced by ACMP (Cavas et al. 2012). This observed divergence may be due to difference in experimental models.

Our findings are important evidence of oxidative stress and lipid peroxidation response generated in liver tissue after ACMP administration. In fact, in this assay, the overproduction of ROS levels and the decrease of antioxidant enzyme activities (SOD, CAT) in the liver is an argument of the failure of antioxidant defense system to eliminate ROS influx.

SOD is the biggest remover of oxygen free radical and the only effective enzyme catalyzing the dismutation reaction of  $O_2^-$  (Wang et al. 2016). In our study, the decreased activity of SOD in the liver can be explained by the consumption of this enzyme in removing  $O_2^-$  into  $H_2O_2$  while CAT, an ubiquitous enzyme, scavenges  $H_2O_2$  to water and oxygen (Tang et al. 2005). However, during these processes, the activities of these antioxidant enzymes involved in free radical could be altered leading to the accumulation of  $H_2O_2$  which promoted lipid

peroxidation in the cell membrane of treated rats manifested by the elevation of the hepatic level of MDA.

## Conclusion

In the light of hematological and biochemical results, histopathological findings, and antioxidant enzyme assessment, it can be concluded from our study that ACMP at 10.85 mg/kg bw has caused minor effects. However, using 21.7 and 43.4 mg/kg bw of commercial product of ACMP causes subchronic hepatotoxicity with a dose-dependent manner. Therefore, because of poor safety procedure, more investigations are utterly recommended concerning risky human exposure to pesticides and potential side impacts on the human health.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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