



# Effect of powdered seed of *Nigella sativa* administration on sub-chronic and chronic lead acetate induced hemato-biochemical and histopathological changes in Sprague Dawley rats

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## Abstract

Lead acetate (PbAC) toxicity can occur by either ingestion or inhalation from contaminated surfaces or from the environment. *Nigella sativa* is a natural product with immense pharmacological properties, which include antioxidant, antibacterial, and anti-anemia properties. It has been showed to counter the effect of PbAC-induced hematological and biochemical changes in short-term studies. This study hypothesized that the *N. sativa* (NS) administration will ameliorate the deleterious effects of chronic PbAC toxicity in rats. A total of 75 Sprague Dawley rats were divided into three groups of 25 rats, and each group was further sub-divided into five groups of five rats each. Group 1 rats (negative control) were given distilled water, group 2 (positive control; PC) were given 10 mg/kg of lead acetate (PbAC) daily, and groups 3 (T1), 4 (T2), and 5 (T3) were each given 10 mg/kg of PbAC followed by graded concentrations of powdered seeds of NS; 100, 150, and 200 mg/kg, respectively. Five rats in each group were euthanized at 30, 60, and 90 days for collection of whole blood and selected organs. Whole blood was collected after euthanized via cardio puncture and used to evaluate the complete blood profile, while plasma was used for biochemical analysis. Tissue samples of the liver and kidney were fixed with 10% buffered formalin, processed, and stained with H&E and periodic acid–Schiff (PAS) for the liver. Aggression and fear were increased in the PbAC-exposed group and absent in the T3 group. There was a lower ( $p < 0.05$ ) red blood cell count (RBC), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), and lymphocyte count in the PC and T1 groups only. Biochemical analysis revealed elevated ( $p < 0.05$ ) liver enzyme and creatinine levels in the PC and T1 groups on day 90 for AST and day 30 for ALT and creatinine. The level of alkaline phosphatase (ALP) was higher ( $p < 0.05$ ) in the PC at 30 and 60 days of sampling. Other parameters, such as WBCs, prothrombin, urea, and cholesterol, were not significant in all groups. Histopathological lesions in the liver and kidneys were more severe in the PC and T1 groups, while the T2 and T3 groups showed mild lesions resulting from *N. sativa* administration. There was a decrease ( $p < 0.05$ ) in the total PAS-stained area signifying glycogen depletion in the PC, T1, and T2 groups at 60 days and a higher distribution of the PAS-stained areas ( $p < 0.05$ ) in the T3 group. At 90 days, the PC group had a lower ( $p < 0.05$ ) distribution of PAS-stained areas in comparison to the other groups. The results showed the therapeutic potential of *N. sativa* extract in modulating both hematological and biochemical alterations induced by chronic lead acetate administration in rats.

**Keywords** *Nigella sativa* · Lead acetate · Hematology · Biochemistry · Histopathology · Sprague Dawley rats

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## Introduction

Lead (Pb) is a common contaminant of the environment and water bodies found in paints, leaded gasoline, dust and soil in gold mining areas, as well as a result of improper industrial waste disposal. Following exposure to Pb, it binds to the blood, soft tissues, and bones, thus remaining in these tissues for a long time. Lead is particularly deadly to young children, resulting in non-regenerative anemia, nervous signs, and development of nutritional deficiencies (Assi et al. 2016a). Adults are mostly exposed at work and show clinical signs of microcytic anemia and nervous symptoms (Papanikolaou et al. 2005). Experimental toxicity studies in laboratory animals have shown various disruptions and alterations in the animal's physiological function. In one study, acute toxicity of lead acetate (PbAC) administered in rats (10 or 100 mg) caused elevation of alanine aminotransferase enzyme (ALT) after 12 and 24 h, respectively (Bharali 2013). In another study, administration of graded doses of PbAC to rats for 30 days showed reduction in total protein, albumin, globulin, total bilirubin, RBC counts, WBC counts, hemoglobin (Hb), and PCV, while increasing the levels of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine (Dalia 2010). Other researchers have found similar findings following chronic administration of PbAC to rats for periods lasting 14–24 weeks (Allouche et al. 2009; Allouche et al. 2011; Ibrahim et al. 2012).

*Nigella sativa* seeds and leaves have been commonly used to alleviate ailments for centuries. Its seeds and oil have been proved to have low toxicity and exhibited good anti-inflammatory, analgesic, anticarcinogenic, antidiabetic, antiulcer, antimicrobial, and antiparasitic activities (Ali and Blunden 2003; Gali-Muhtasib et al. 2006). Animal studies have shown the protective effects of *N. sativa* against several toxic compounds, such as isoproterenol, tramadol, carbon tetrachloride, cisplatin, and sodium valproate. The protective effect of *N. sativa* in these studies was attributed to the regulation of oxidative enzyme levels, hepatic enzyme activities, renal function markers, and blood lipid profiles (Hala 2011; Awadalla 2012; Murugesan et al. 2012; Krishnan and Muthukrishnan 2012; Elkhateeb et al. 2015). In another study, *N. sativa* seeds were shown to ameliorate the biochemical imbalances and tissue damage associated with PbAC toxicity in rats (Farrag et al. 2007). However, the study lasted for only 6 weeks and did not reflect the true picture associated with chronic PbAC toxicity. Currently, there is a paucity of information on the effects of powdered seeds of *N. sativa* administration in chronic PbAC toxicity in rats. Thus, in this study, we investigated the effect of PbAC toxicity for 90 days and how concurrent administration of graded doses of NS counteracted these effects in the blood, serum, and tissues.

## Materials and methods

### Preparation of *N. sativa* seed suspension and lead acetate solution

Black seeds (*N. sativa*) were purchased from a local market in Serdang, Selangor, Malaysia, certified, cleaned, and prepared into powder form as described earlier (Assi et al. 2017a). Lead acetate (Oxford Lab. Co., India) was also prepared at a concentration of 10 mg/kg body weight in distilled water as previously described (Assi et al. 2017a).

### Animal management, grouping, and clinical observation

In this study, 75 male Sprague Dawley rats aged 8–10 weeks and 250–300 g were used. The rats were acclimatized for 2 weeks before the commencement of the experiment. They were fed standard pellet commercial rat chow (Gold Coin, Malaysia), and clean water was provided ad libitum. Before the experimentation, the rats were grouped into five groups of 15 animals each as shown in Table 1.

Clinical monitoring of the rats was done every day. However, important observations, such as aggression, fear, and reduced appetite, were summarized at the end of each sampling period and presented in percentages based on the number of rats showing such signs within a group.

### Sample collection for hematology, biochemistry, and histopathology

Following anesthesia at stipulated periods (30, 60, and 90 days), whole blood was collected intracardially from five animals per group and stored in EDTA-laced tubes (Weisbroth et al. 2013). Plasma was obtained following centrifugation of the blood at 2500 rpm for 10 min (Hettich, Germany). Tissue samples of the liver and kidneys were collected and fixed in 10% neutral buffered formalin.

### Evaluation of hematological and biochemical parameters

Whole blood was used for estimation of hematological parameters using an Automatic Cell Counter (CELL-DYN 3700, Roche, USA). Biochemical parameters for the serum obtained from whole blood were evaluated with an automated chemistry analyzer (Siemens, USA) as described earlier (Assi et al. 2017b).

### Histopathology

Formalin-fixed tissues were processed routinely before embedding in paraffin wax. Tissue blocks were then sectioned at a

**Table 1** Tabular representation of the methodology

Animal grouping ( <i>n</i> = 75)	Sampling period (days)		
	30 days	60 days	90 days
<i>Control</i> ( <i>n</i> = 15), given distilled water orally, daily <i>Positive control</i> ( <i>n</i> = 15), given lead acetate (10 mg/kg) orally, daily (Mustafa and Hussein 2016)	5 rats from each group were euthanized for blood and tissue sample collection on each day of sampling stated above.		
<i>Treatment 1</i> ( <i>n</i> = 15), given NS (100 mg/kg) (Kocyigit et al. 2009) and LA (10 mg/kg), orally, daily (Mustafa and Hussein 2016)			
<i>Treatment 2</i> ( <i>n</i> = 15), given NS (150 mg/kg) (Murugesan et al. 2012) and LA (10 mg/kg), orally, daily (Mustafa and Hussein 2016)			
<i>Treatment 3</i> ( <i>n</i> = 15), given NS (200 mg/kg) (Kocyigit et al. 2009) and LA (10 mg/kg), orally, daily (Mustafa and Hussein 2016)			

thickness of 5–6  $\mu\text{m}$  and stained with hematoxylin and eosin for histopathological examination of lesions with light microscopy. Photomicrographs of tissue microscopic focal fields were captured using Mioticam® camera. Histopathological lesion scoring was done as described earlier, using a scale of 0–3 for lesion severity (Othman et al. 2016). Six microscopic focal fields were examined per slide using 400 $\times$  magnification, and the lesion scores equated as 0–3.

The periodic acid–Schiff (PAS) staining was performed on the sectioned slides. Briefly, the slides were inserted in a container of 0.5% periodic acid for 5 min, followed by three wash cycles using distilled water. The slides were placed into a solution of Schiff's reagent for 15 min, then washed in 0.55% metabisulfite twice for 1 min each, followed by washing in running tap water for 10 min for bluing. Acidified Harris hematoxylin was used to counterstain the slides for 30 s followed by washing the slides in water. The sections were dehydrated in 95–100% alcohol then xylene before mounting with coverslips and viewing using light microscopy. The presence of rose pink or magenta coloration within the hepatocytes was used as an indication of glycogen deposition or presence of polysaccharides.

The PAS-stained areas were estimated used ImageJ software as previously described by Rangan and Tesch (2007). Briefly, the images were opened with the ImageJ software and converted to grayscale. The image threshold is then set from an image montage and adjusted while viewing the histogram in order to ensure a precise selection of the stained areas. Once the threshold is set, the image is converted to binary (black and white), and the stained areas ( $\text{mm}^2$ ) were determined and recorded as mean  $\pm$  S.E. by using the analyze option.

### Statistical analysis

Data obtained from hematology, biochemistry, and histopathology scores were summarized as mean  $\pm$  SE and analyzed

with the aid of GraphPad Prism 6.0 software using two-way analysis of variance (ANOVA) with Bonferroni's multiple comparison test. Comparison between histopathological scores was done using Kruskal–Wallis (non-parametric) test with Dunn's multiple comparison. Statistical significance was set at  $p < 0.05$ .

## Results

### Clinical observations

The mortality rate at 30 days was 7% ( $n = 15$ ) in the PC, T1, and T2 groups, while none was observed in the T3 group. At 60 days, mortality was 10% ( $n = 10$ ) in the PC group only, while no mortality was recorded in all groups at 90 days of sampling. The main clinical observations, such as aggression, fear, and loss of appetite, were observed to be higher in the PC and T1 groups at 30 days of sampling, while the T2 had a lower percentage and the T3 had none. At 60 days, aggression and fear were higher in the PC group, while reduced appetite was comparable and higher in both PC and T1 groups. Similarly, at 90 days, the PC group had higher values of all observations followed by the T1, while the T2 had a lower percentage (Table 2).

### Hematology findings

The total red blood cell (RBC) count was lower ( $p < 0.05$ ) in the positive control (PC) group on days 30, 60, and 90 of sampling with no changes in all treatment groups at all time periods. There was no significant change in hemoglobin concentration in the entire days of sampling. The PCV was lower ( $p < 0.05$ ) in the PC group on days 30 and 60, while no changes were observed at 90 days. There was no difference in the MCV values of all groups on days 30 and 60, while at 90 days,

**Table 2** Summary of clinical observations (%) made in rats following PbAC and *N. sativa* administration at 30, 60, and 90 days of experimentation

Groups/signs	Aggression (%)	Reduced appetite (%)	Fear (%)
30 days			
PC	80	100	100
T1	80	100	100
T2	40	20	20
T3	0	0	0
60 days			
PC	80	100	80
T1	60	100	60
T2	40	20	20
T3	30	0	0
90 days			
PC	100	60	80
T1	80	60	60
T2	40	20	20
T3	0	0	0

PC, positive control; T1, treatment group one given PbAC 10 mg/kg and *N. sativa* 100 mg/kg; T2, treatment group two given PbAC 10 mg/kg and *N. sativa* 150 mg/kg; T3, treatment group three given PbAC 10 mg/kg and *N. sativa* 200 mg/kg

both PC and treatment groups had lower MCV values when compared to the control group. The MCHC value was lower in the PC and T1 groups at day 30 of sampling. However, on day 60, both the PC and treatment groups had a lower MCHC value than the control, while no changes were observed at 90 days of sampling. There was no significant change in prothrombin concentration on entire days of sampling (Fig. 1).

There was no significant change in total white blood cell, monocytes, and eosinophil counts in all groups in entire days of sampling. However, the lymphocyte count was lower ( $p < 0.05$ ) in the T1 group on day 30 with no changes on subsequent sampling periods. Similarly, neutrophil count was higher in the PC group on day 30 without any changes on days 60 and 90. The thrombocyte count was comparable in all groups on days 30 and 90, while in the T3 group, it was lower ( $p < 0.05$ ) on day 60 (Fig. 2).

### Biochemical findings

The level of AST activity was unchanged in all groups on days 30 and 60 of sampling. On day 90, AST activity was elevated in the PC and T1 groups when compared to the control group. On the other hand, ALT activity was elevated in the PC and T1 groups on day 30 of sampling, while no changes were observed on days 60 and 90. Similarly, ALP activity was higher ( $p < 0.05$ ) in the PC group on days 30 and 60, with no changes on day 90. The concentration of creatinine was higher ( $p < 0.05$ ) in the PC and T1 groups on day 30 of sampling

with no changes seen on days 60 and 90. There were no significant changes in cholesterol and urea concentration in the entire days of sampling (Fig. 3).

### Histopathological findings

The histopathological lesion scores of the liver and kidney are presented in Table 3. At day 30 of sampling, tubular degeneration (TD) in the kidneys was higher ( $p < 0.05$ ) in the PC group than in the T1 group. T2 and T3 groups had TD comparable with the control group. Hepatocyte vacuolation (HV), Kupffer cell proliferation (KCP), and pyknotic hepatocytes (PH) were higher in the PC and T1 groups at 30 days of experiment.

At 60 days of sampling, TD and HV were higher ( $p < 0.05$ ) in PC and T1 groups, while KCP and PH were higher in PC group alone.

At day 90 of sampling, TD and HV were comparable and low ( $p < 0.05$ ) in all PbAC exposed and treated groups. However, KCP was higher ( $p < 0.05$ ) in the PC and T1 groups, while PH was comparable and higher ( $p < 0.05$ ) in PC, T1, and T2 groups (Figs. 4 and 5).

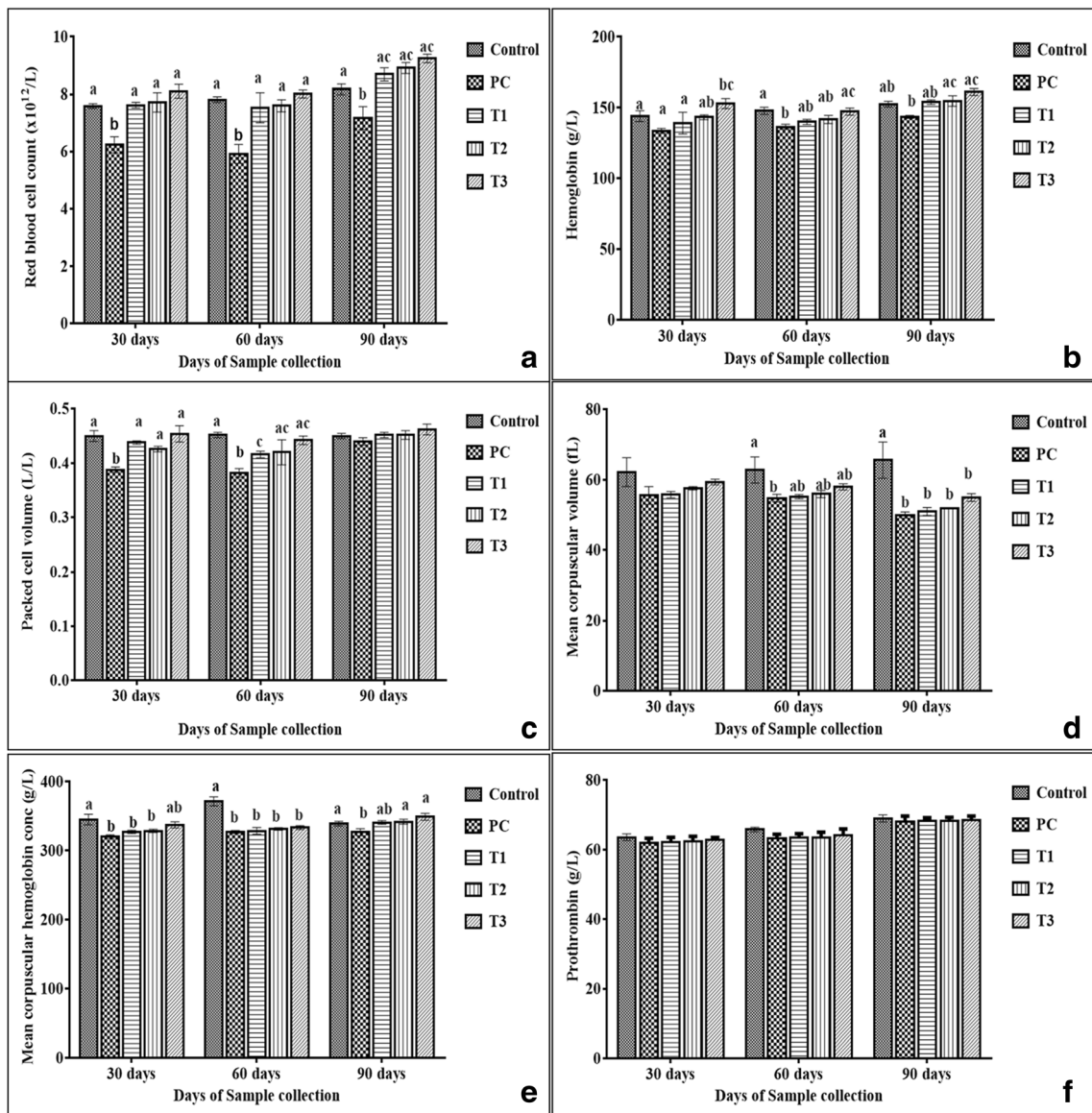
### Periodic acid–Schiff staining

The mean  $\pm$  SE of PAS-stained areas in the liver of the various groups is shown in Table 4. The PAS-stained section of the liver at 30 days showed uneven magenta staining of mucopolysaccharides within the hepatocytes of all groups examined. However, there was no difference ( $p > 0.05$ ) in the distribution of PAS-positive areas in all the groups (Fig. 6). At day 60 post-treatment, the PC, T1, and T2 groups had lower distribution of PAS-positive areas in comparison to the control, which is an indicative of glycogen depletion within the hepatocytes. However, the T3 group showed a diffuse and higher ( $p < 0.05$ ) distribution of PAS-positive areas in the liver (Fig. 6). After 90 days of treatment, the distribution of PAS-positive areas was comparable in the control and all the treatment groups. However, the PC group showed a lower ( $p < 0.05$ ) distribution of PAS-stained areas in comparison to the control and treatment groups, indicative of mild glycogen depletion in the hepatocytes of this group (Fig. 6).

### Discussion

Lead acetate has been shown to induce neurological dysfunction in lab animals. Some of these dysfunctions include cognitive impairment, aggression, slower acquisition, and visual discrimination (Driscoll and Stegner 1976). However, the dose and duration of PbAC exposure and age at which the animals were exposed significantly affected these dysfunctions. For example, the administration of 0.5% PbAC to older





**Fig. 1** Graphs showing hematological changes in rats exposed to PbAc and treatment groups. **a** RBC count, **b** hemoglobin concentration, **c** packed cell volume, **d** mean corpuscular volume, **e** mean corpuscular

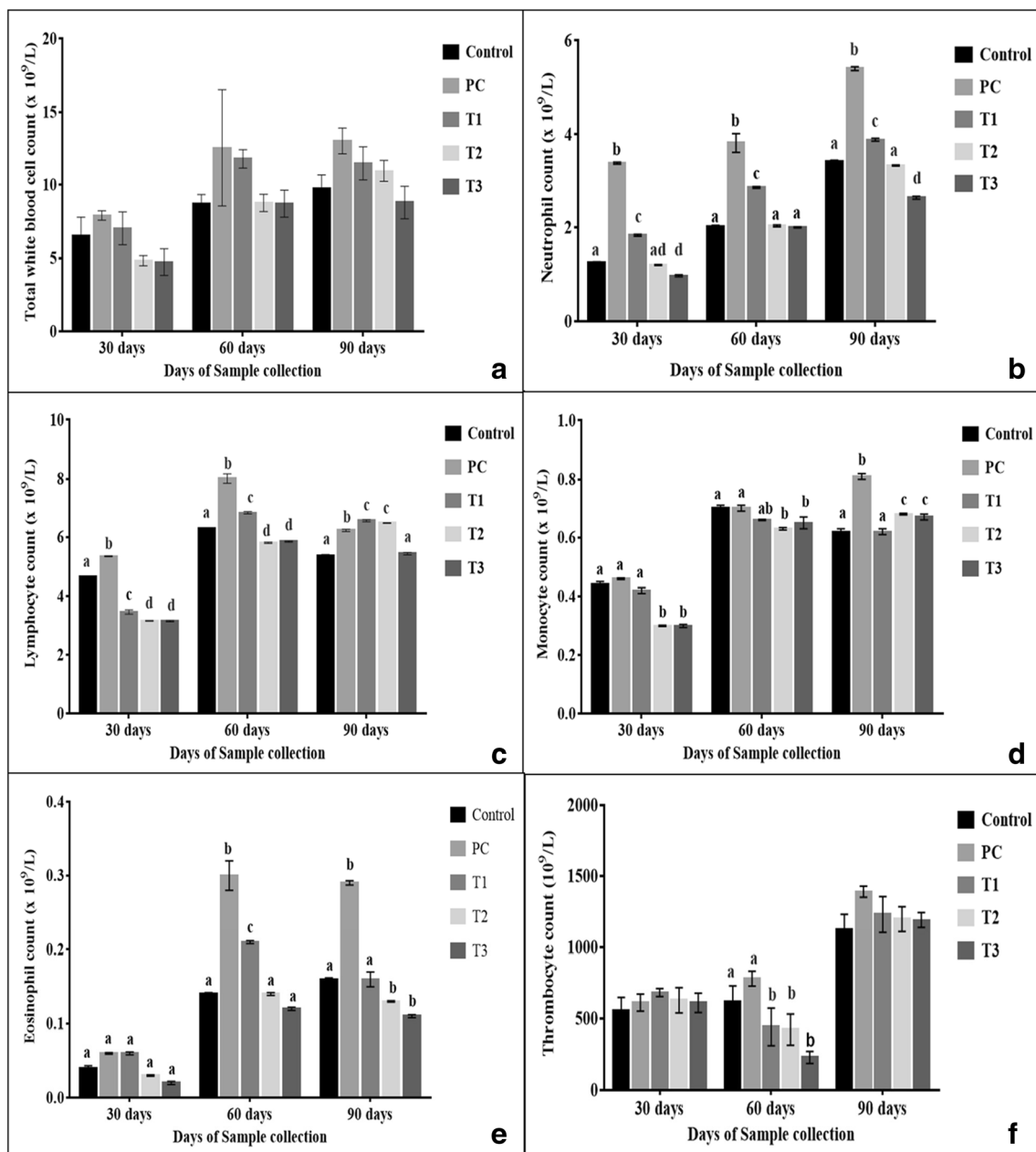
hemoglobin concentration, and **f** prothrombin concentration. <sup>a,b,c</sup>Bars with different superscript letters indicate statistical significance at  $p < 0.05$

mice (330 and 660 days old) increased aggression when compared with administration to younger mice (65 and 120 days) (Burrigh et al. 1983; Engellenner et al. 1986). In this study, we observed increased aggression and fear in animals exposed to PbAC, while those treated with low-dose powdered seeds of *N. sativa* had lower incidence of these signs. Interestingly, rats treated with 200 mg/kg of *N. sativa* did not show signs of aggression and fear during all sampling periods, thus suggesting that the powdered seeds of *N. sativa* prevented the manifestations of these signs.

In this study, PbAC induced decrease in RBCs and PCV counts in the PC group on 30 and 60 days of treatment, and these effects were reversed by *N. sativa* seed powder administration in treated animals. This shows that *N. sativa* seeds

can prevent anemia induced by PbAC. Similarly, decrease in lymphocytes and increase in neutrophil counts in the PC and T1 groups were observed, both of which were reversed by *N. sativa* seed powder administration in the treatment groups. These results supported the earlier toxicity studies showing the negative effects of PbAC on the hemogram of laboratory animals (Dalia 2010; Ibrahim et al. 2012), adding to the therapeutic effects of *N. sativa* in improving the hemogram as previously described (Ali and Blunden 2003; Gali-Muhtasib et al. 2006).

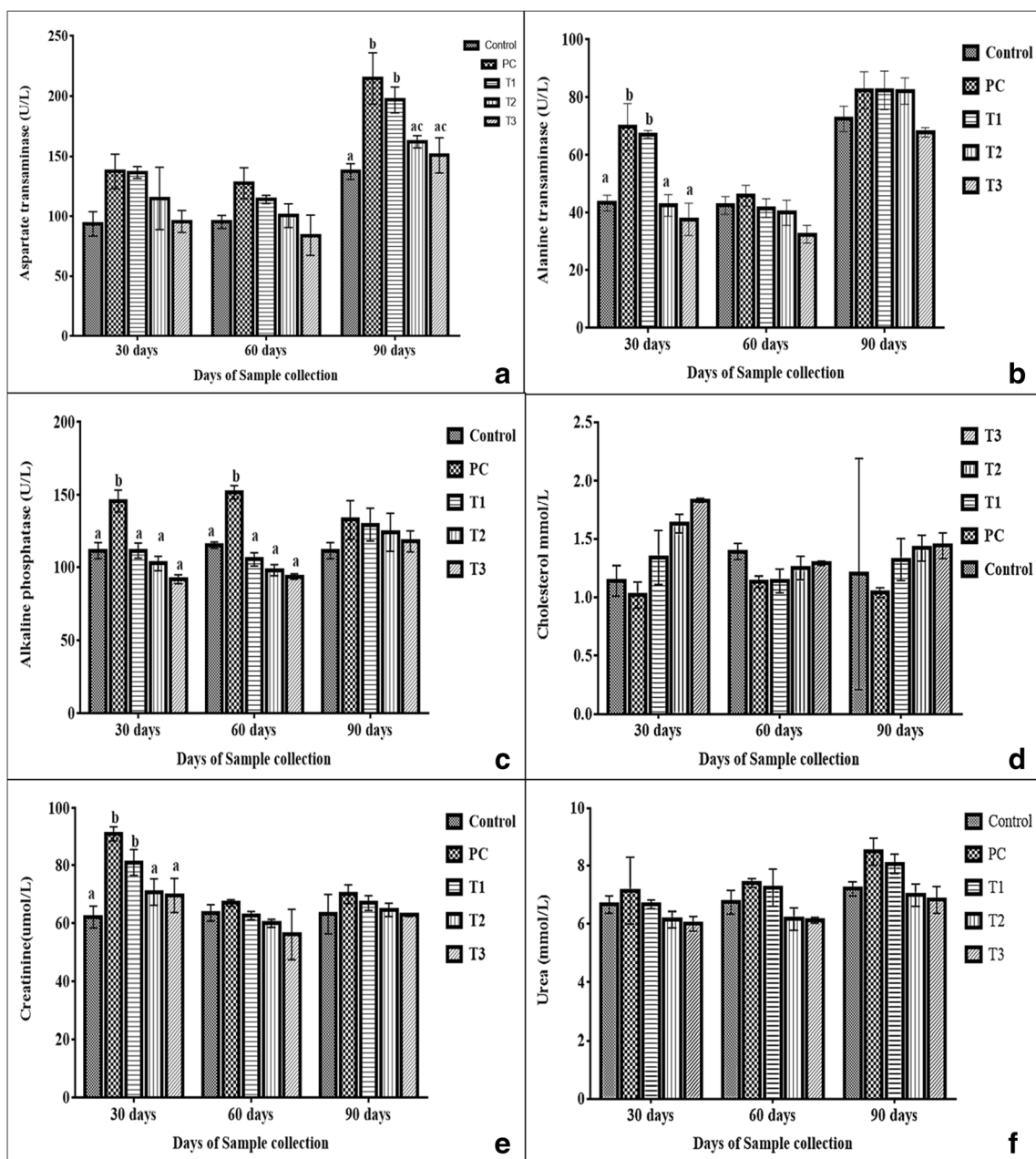
Another pertinent finding observed in this study was the elevated levels of ALT and ALP in the PC group at 30 and 60 days of sampling and increased AST at 90 days of sampling. ALT and AST are two important enzymes that signify



**Fig. 2** Graphs showing hematological changes in rats exposed to PbAc and treatment groups. **a** WBC count, **b** neutrophil count, **c** lymphocyte count, **d** monocyte count, **e** eosinophil count, **f** thrombocyte count. <sup>a,b,c</sup> Bars with different superscript letters indicate statistical significance at  $p < 0.05$

liver function in animals. An increased level of these enzymes indicates possible hepatocellular damage or injury. Increased levels of these enzymes have been previously reported following PbAC toxicity in rats (Allouche et al. 2011; Ibrahim et al. 2012; Bharali 2013). In these studies, elevated levels of AST, ALT, and ALP were observed following acute and chronic PbAC administration to rats. Furthermore, we also observed that powdered seeds of *N. sativa* acted as a hepatoprotective agent since it regulates the levels of ALT, AST, and ALP in treated rats. Similarly, based on our histopathological findings, the PbAC-exposed group had a higher distribution of

hepatocyte degeneration and necrosis, which was responsible for the increased enzyme levels observed. On the other hand, the treated groups had lower distribution of these pathological lesions, suggesting a hepatoprotective effect of the seed extract against PbAC-induced liver damage. In our previous report, we showed evidence of decreased GSH and SOD enzyme levels in rats exposed to PbAC. We also showed increased levels indicative of antioxidant capacity in rats treated with powdered seeds of *N. sativa* especially at 200 mg/kg body weight (Assi et al. 2016b). Presence of PbAC in the hepatocytes has been reported to induce necrosis,



**Fig. 3** Graphs showing biochemical changes in rats exposed to PbAc and treatment groups. **a** Aspartate transaminase, **b** alanine transaminase, **c** alkaline phosphatase, **d** cholesterol, **e** creatinine, **f** urea. <sup>a,b,c</sup>Bars with different superscript letters indicate statistical significance at  $p < 0.05$

degeneration, and fatty change in a dose-dependent manner (Mudipalli 2007). In this study, the use of PAS stain in the liver sections revealed the presence of glycogen depletion in the PC, T1, and T2 groups, especially at 60 days of sampling. Glycogen depletion has been shown to be one of the toxic effects of LA in the liver. In a study by Hegazy and Fouad (2014), mild and moderate glycogen depletions were observed in rats administered 0.13% lead acetate after 4 and 8 weeks, respectively. Although there are no studies on the effect of *N. sativa* administration on LA-induced glycogen depletion in the liver, a related study by Al-Rouby and Gawish (2013) showed unaltered glycogen deposits in the liver of rats treated

with *N. sativa* oil following exposure to sodium valproate. Based on this limited information, it can be deduced that *N. sativa* seeds prevent damage to hepatocytes which thus prevents depletion of their glycogen stores. *N. sativa* has been shown to restore deficits in oxidative enzyme levels, such as GSH and SOD in the cell following exposure to oxidant compounds, such as PbAC, which often results in lower concentration of these enzymes (Salem 2005). In a study by Farrag et al. (2007), rats exposed to PbAC for 6 weeks were observed to have elevated levels of AST, which was ameliorated by administration of *N. sativa* seeds. However, the study was only able to show the sub-chronic effects of *N. sativa*

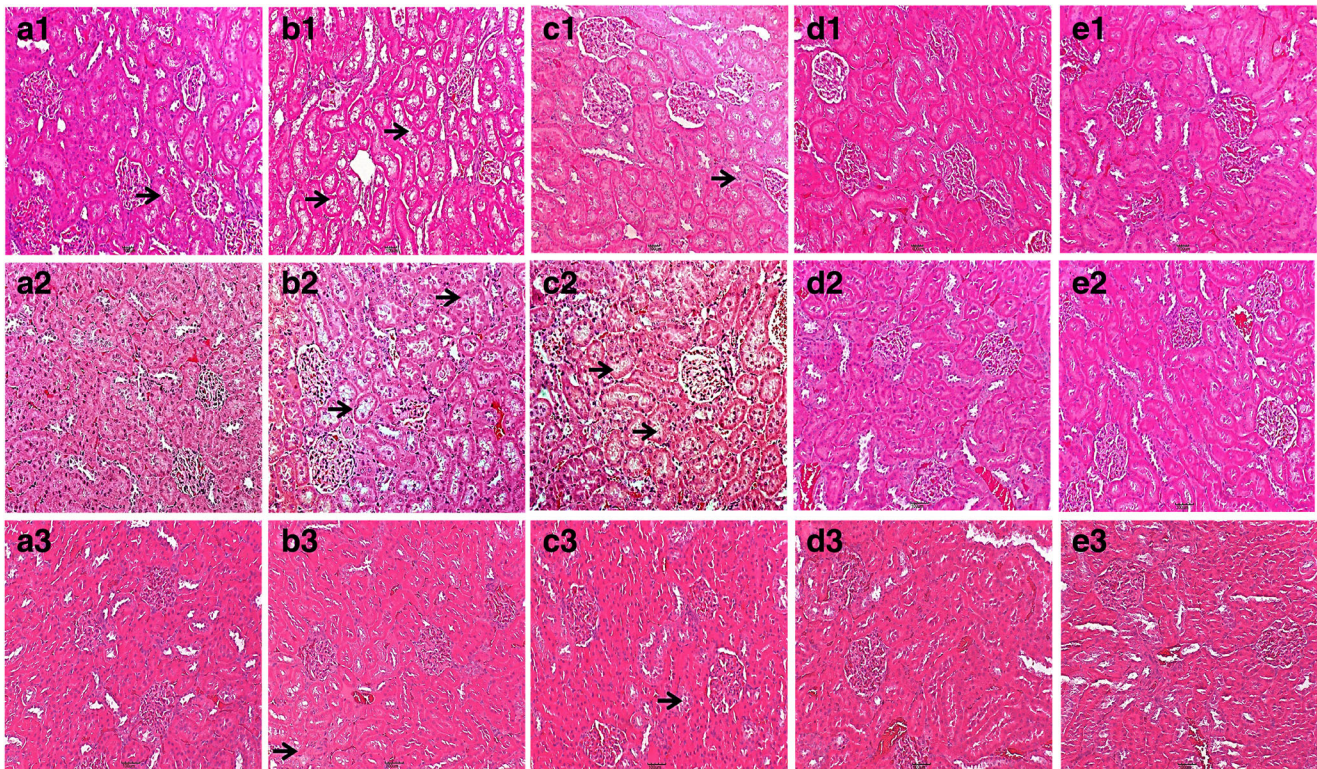


**Table 3** Summary of histopathological lesion scores (mean  $\pm$  S.E.M.) in the kidney and liver of rats exposed to PbAc and treated with *N. sativa*

	Grouping				
	NC	PC	T1	T2	T3
Histopathological lesions	30 days				
Tubular degeneration	0.2 <sup>a</sup> $\pm$ 0.0	2.2 <sup>b</sup> $\pm$ 0.3	0.5 <sup>c</sup> $\pm$ 0.1	0.3 <sup>ac</sup> $\pm$ 0.1	0.2 <sup>a</sup> $\pm$ 0.0
Hepatocyte vacuolation	0.3 <sup>a</sup> $\pm$ 0.1	1.5 <sup>b</sup> $\pm$ 0.3	1.6 <sup>b</sup> $\pm$ 0.3	1.0 <sup>c</sup> $\pm$ 0.1	0.5 <sup>a</sup> $\pm$ 0.1
Kupffer cell proliferation	0.0 <sup>a</sup> $\pm$ 0.0	1.1 <sup>b</sup> $\pm$ 0.1	1.0 <sup>b</sup> $\pm$ 0.1	0.5 <sup>c</sup> $\pm$ 0.2	0.3 <sup>c</sup> $\pm$ 0.1
Pyknotic hepatocytes	0.0 <sup>a</sup> $\pm$ 0.0	1.2 <sup>b</sup> $\pm$ 0.2	1.0 <sup>b</sup> $\pm$ 0.2	0.3 <sup>c</sup> $\pm$ 0.1	0.3 <sup>c</sup> $\pm$ 0.1
	60 days				
Tubular degeneration	0.2 <sup>a</sup> $\pm$ 0.1	1.6 <sup>b</sup> $\pm$ 0.2	1.5 <sup>b</sup> $\pm$ 0.3	0.3 <sup>a</sup> $\pm$ 0.1	0.2 <sup>a</sup> $\pm$ 0.1
Hepatocyte vacuolation	0.2 <sup>a</sup> $\pm$ 0.1	0.7 <sup>b</sup> $\pm$ 0.2	0.6 <sup>b</sup> $\pm$ 0.1	0.2 <sup>a</sup> $\pm$ 0.1	0.2 <sup>a</sup> $\pm$ 0.1
Kupffer cell proliferation	0.0 <sup>a</sup> $\pm$ 0.0	1.0 <sup>b</sup> $\pm$ 0.1	0.5 <sup>c</sup> $\pm$ 0.2	0.3 <sup>c</sup> $\pm$ 0.1	0.2 <sup>c</sup> $\pm$ 0.1
Pyknotic hepatocytes	0.0 <sup>a</sup> $\pm$ 0.0	0.5 <sup>b</sup> $\pm$ 0.0	0.3 <sup>c</sup> $\pm$ 0.1	0.2 <sup>c</sup> $\pm$ 0.1	0.2 <sup>c</sup> $\pm$ 0.1
	90 days				
Tubular degeneration	0.0 <sup>a</sup> $\pm$ 0.0	0.3 <sup>b</sup> $\pm$ 0.1	0.2 <sup>b</sup> $\pm$ 0.1	0.2 <sup>b</sup> $\pm$ 0.1	0.2 <sup>b</sup> $\pm$ 0.1
Hepatocyte vacuolation	0.0 <sup>a</sup> $\pm$ 0.0	0.5 <sup>b</sup> $\pm$ 0.2	0.5 <sup>b</sup> $\pm$ 0.2	0.3 <sup>b</sup> $\pm$ 0.1	0.3 <sup>b</sup> $\pm$ 0.1
Kupffer cell proliferation	0.0 <sup>a</sup> $\pm$ 0.0	1.2 <sup>b</sup> $\pm$ 0.2	1.0 <sup>b</sup> $\pm$ 0.2	0.5 <sup>c</sup> $\pm$ 0.1	0.5 <sup>c</sup> $\pm$ 0.1
Pyknotic hepatocytes	0.0 <sup>a</sup> $\pm$ 0.0	1.5 <sup>b</sup> $\pm$ 0.2	1.2 <sup>b</sup> $\pm$ 0.2	1.0 <sup>b</sup> $\pm$ 0.2	0.5 <sup>c</sup> $\pm$ 0.1

a, b, c Signifies statistical significance at  $p < 0.05$

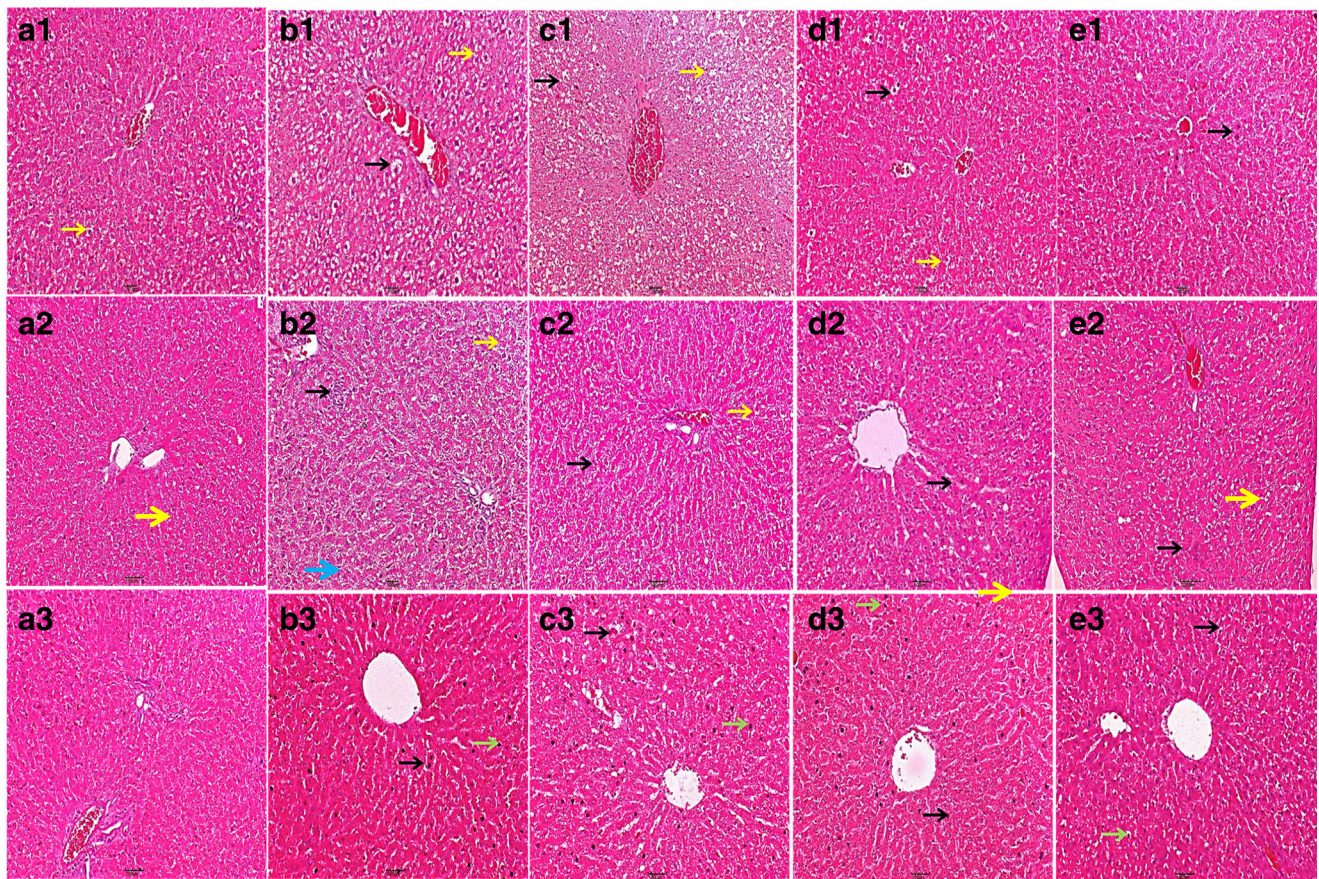
0, no lesion; 1, mild lesion (<30%); 2, moderate (<60%); 3, severe (>60%)



**Fig. 4** Photomicrograph of the kidneys of rats at day 30 of sample collection. **a1** NC showing mild vacuolation of the renal tubular cells, **b1** PC showing moderate vacuolation of the renal tubular cells, **c1** T1 showing mild vacuolation of the renal tubular cells, **d1** T2, and **e1** T3 not having obvious vacuolation of the renal tubular cells (arrows); 60 days of sample collection. **a2** NC, **b2** PC showing moderate vacuolation of the

renal tubular cells, **c2** T1 showing mild vacuolation of the renal tubular cells, **d2** T2, and **e2** T3 not having obvious vacuolation of the renal tubular cells; 90 days of sample collection. **a3** NC, **b3** PC, **c3** T1, **d3** T2, and **e3** T3; note mild and occasional vacuolation of the renal tubular cells in B3 and C3, H&E 200 $\times$





**Fig. 5** Photomicrograph of the liver of rats at 30 days of sample collection. **a1** NC showing mild vacuolation of the hepatocytes, **b1** PC showing moderate vacuolation of the hepatocytes (yellow arrows) and pyknotic hepatocytes cells (black arrow), **c1** T1 showing moderate vacuolation of hepatocytes (yellow arrow) and mild hepatocyte pyknosis (black arrow), **d1** T2 and **e1** T3 showing mild hepatocyte vacuolation (yellow arrow) and pyknotic hepatocytes (black arrow); 60 days of sample collection. **a2** NC showing occasional mild vacuolation of the hepatocytes (yellow arrow), **b2** PC showing an area

of leucocytic infiltration (black arrow), moderate vacuolation of the hepatocytes (yellow arrow), and pyknotic hepatocytes (blue arrow). **c2** T1 showing moderate vacuolation of hepatocytes. **d2** T2 and **e2** T3 showing mild hepatocyte vacuolation (yellow arrow) and nuclear pyknosis (black arrow); 90 days of sample collection. **a3** NC showing apparently normal liver tissue with a congested central vein, **b3** PC, **c3** T1, **d3** T2, and **e3** T3 showing pyknotic hepatocytes (black arrow) and numerous active Kupffer cells (green arrows), H&E 200×

treatment on PbAC induced toxicity, while our study evaluated the sub-chronic and chronic effects altogether. Furthermore, we observed that both ALP and ALT were not elevated in all groups at 90 days, while AST was higher in the PC and T1 groups only. This may be attributed to

increased excretion of lead in the bile and intestines with less retention in the liver, hence resulting in less hepatocyte necrosis as the time of exposure was prolonged. Since the dose of PbAC reported to cause inhibition of CYP450 and cytochrome b5 in the liver and kidney was 50 mg/kg, there

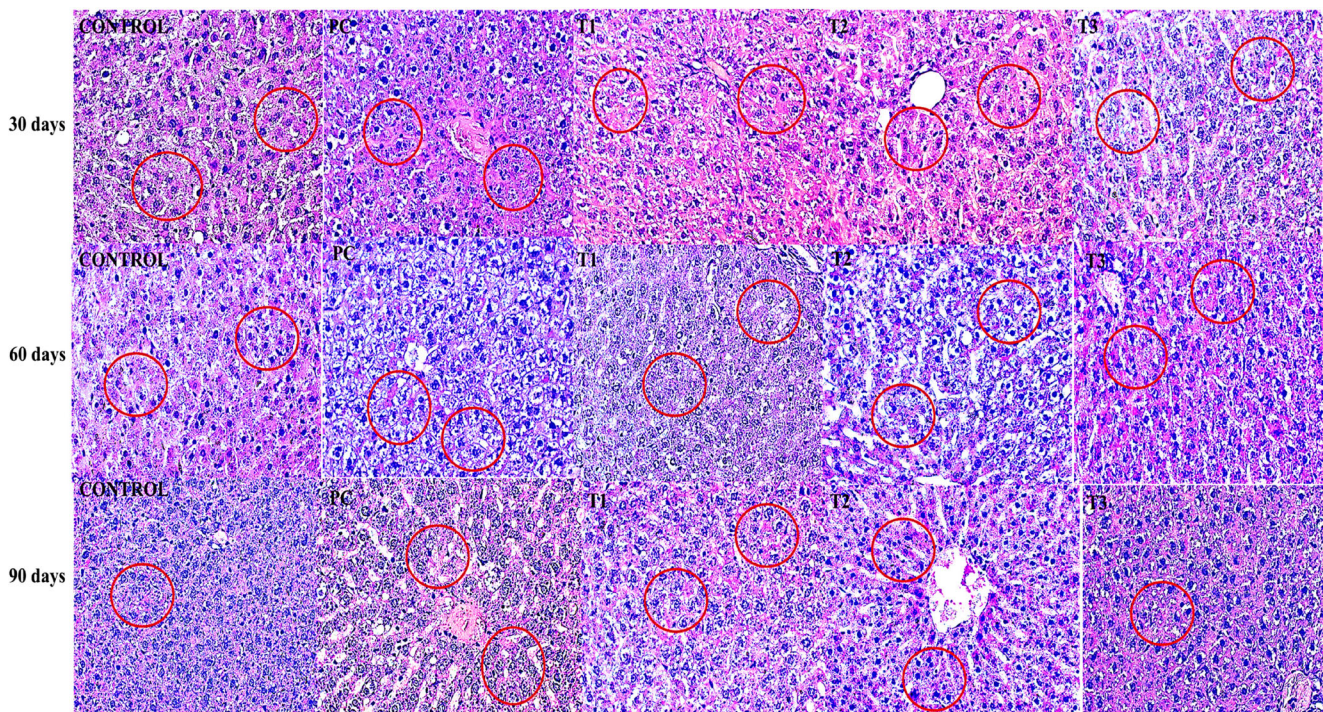
**Table 4** Area of rat hepatic tissue positive for periodic acid–Schiff (PAS) staining after exposure of PbAC and treatment with *Nigella sativa* after 30, 60, and 90 days

Treatment	Stained area (mm <sup>2</sup> )		
	30 days	60 days	90 days
Control	1.31 ± 0.2 × 10 <sup>3</sup>	1.89 <sup>a</sup> ± 0.3 × 10 <sup>3</sup>	2.51 <sup>a</sup> ± 0.3 × 10 <sup>3</sup>
Positive control	1.29 ± 0.3 × 10 <sup>3</sup>	1.47 <sup>b</sup> ± 0.3 × 10 <sup>3</sup>	1.79 <sup>b</sup> ± 0.1 × 10 <sup>3</sup>
T1	1.30 ± 0.2 × 10 <sup>3</sup>	1.44 <sup>b</sup> ± 0.5 × 10 <sup>3</sup>	2.54 <sup>a</sup> ± 0.3 × 10 <sup>3</sup>
T2	1.32 ± 0.3 × 10 <sup>3</sup>	1.40 <sup>b</sup> ± 0.3 × 10 <sup>3</sup>	2.56 <sup>a</sup> ± 0.2 × 10 <sup>3</sup>
T3	1.35 ± 0.4 × 10 <sup>3</sup>	2.36 <sup>c</sup> ± 0.4 × 10 <sup>3</sup>	2.59 <sup>a</sup> ± 0.5 × 10 <sup>3</sup>

All the values are expressed as the mean ± standard error of the mean

<sup>a,b,c</sup> Significant differences (*p* < 0.05) compared with normal control





**Fig. 6** Section of the liver of rats in different treatment groups showing PAS-positive areas indicating presence of mucopolysaccharide within the hepatocytes. There is no difference in the distribution of PAS-positive

areas in all groups at 30 days. Note lower distribution of PAS-positive areas in the PC, T1, and T2 at 60 days; the PC group showed lower PAS-stained areas than the other groups, PAS stain 400 $\times$

may be minimal to low inhibition of these enzymes in rats in this study (Mudipalli 2007).

In this study, the kidney function was not severely affected by PbAC toxicity, since elevated levels of creatinine were only observed at 30 days of sampling in both PC and T1 groups. Other studies using PbAC at graded doses also observed similar or more severe increases in kidney enzymes, such as creatinine and urea (Farrag et al. 2007; Dalia 2010; Elkhateeb et al. 2015). Exposure to high levels of lead has been shown to induce acute and chronic nephropathy characterized by increased renal biochemical parameters. This has been attributed to the development of degenerative changes in the renal tubules and presence of nuclear inclusion bodies containing lead complexes (Rastogi 2008). In this study, treatment of rats with 150 and 200 mg/kg of powdered seeds of *N. sativa* prevented the increase in creatinine in blood on day 30 of PbAC toxicity, suggesting a possible nephro-protective effect by the seed extract of *N. sativa*. The nephro-protective effect of *N. sativa* has been previously reported following PbAC, gentamycin, and tramadol nephron-toxicities (Farrag et al. 2007; Yaman and Balikci 2010; Saleem et al. 2012; Elkhateeb et al. 2015). In these studies, *N. sativa* was shown to ameliorate nephrotoxicity induced by these compounds due to a restoration of the imbalance of oxidative enzyme levels.

Furthermore, in this study, PbAC induced histopathological lesions in both the kidney and liver. The lesions observed appeared more severe in the PC and T1 groups and mild in the

treated groups, indicating that treatment with powdered seeds of *N. sativa* reduced and eventually reversed these injuries at 150 and 200 mg/kg. PbAC has been previously shown to induce increased vacuolation in the liver of rats 24 h after administration of 100 mg/kg (Bharali 2013) of PbAC. In other related studies, PbAC has also been shown to induce severe damages to the kidney and liver after 4–6 weeks of administration (Farrag et al. 2007; Dalia 2010). In the study by Farrag et al. (2007), administration of *N. sativa* to the PbAC-intoxicated rats improved the histopathological lesions in the kidney and liver. This finding substantiates with our current results since we observed a restoration of normal enzyme levels and tissue morphology in both the kidney and liver after 90 days of PbAC and *N. sativa* seed powder administration, especially 200 mg/kg *N. sativa*. Similarly, our recent study on the prophylactic activity of *N. sativa* showed promising results against PbAC-induced toxicity by preventing the development of hemato-biochemical and histopathological alterations in rats (Assi et al. 2017b).

## Conclusion

To conclude, this study showed that chronic PbAC administration to rats induced hematological, biochemical, and histopathological changes. The concurrent administration of powdered seeds of *N. sativa* at 150 and 200 mg/kg was able to



reverse the alterations induced by PbAC in sub-chronic and chronic conditions.

**Author contribution** All authors contributed equally to this work.

## Compliance with ethical standards

**Ethical statement** The animal experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee (IACUC) with reference number UPM/IACUC/AUP-R047/2015, in accordance with the standard guidelines on usage and care of laboratory animals. The animals were humanely handled and euthanized at stipulated dates (30, 60, and 90 days) during the experimental period, using CO<sub>2</sub> asphyxiation method after anesthesia procedure with ketamine + xylazine (50 mg/kg + 10 mg/kg body weight).

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

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