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Protective Effect of *Nigella sativa* Seeds Against Lead-induced Hepatorenal Damage in Male Rats

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Abstract: Heavy metals are widely distributed in the environment and some of them occur in food, water, air and tissues even in the absence of occupational exposure. Among of these lead, (Pb) is a hazardous substance to human and animal's. The present study was carried out to investigate the possible protective effect of co-administered *Nigella sativa* seeds on lead acetate-induced rats' toxicity in particularly on liver and kidney. Thirty-six male rats were divided into six groups, 6 rats each. The first group was served as a control, while the second group was fed on the basal diet with *Nigella sativa* addition, whereas the other groups contained lead acetate (10 and 20% of LD_{50}) with and without *Nigella sativa* supplementation for six weeks. At the end of the feeding period, rats were fasted over night and anesthetized and blood and tissue samples were taken for biochemical and histopathological studies. The results of this study revealed that lead acetate caused significant elevations in AST, urea, creatinine, total cholesterol and triglycerides in serum. Lead treatment also produced significant decrease in serum total protein and albumin. Histopathological observations showed severe damage in the liver and kidneys. Its damaged areas were measured using Image analyzer. Combined treatment of lead-exposed animals with *Nigella sativa* showed marked improvement in both biochemical and histopathological findings as well as reduction in the damaged areas. These experimental results strongly indicate the protective effect of *Nigella sativa* against toxic effect of lead on liver and kidney tissues.

Key words: Lead acetate, *Nigella sativa*, biochemical, histopathological, rats

INTRODUCTION

Lead (Pb) and its compounds play a significant role in modern industries; a wide variety of population were at risk of occupational exposure and lead is suspected to be a human carcinogen (Fracasso *et al*., 2002).

Lead poisoning causes renal dysfunction, liver cirrhosis, damage to the central nervous system and anemia (Sheffield *et al*., 2001; Damek-Poprawa and Sawicka-Kapusta, 2004). Lead administration also exerted toxic actions in other organs (Oberley *et al*., 1995; Flora and Seth, 2000; Villeda-Hernandez *et al*., 2001; El-Sokkary *et al*., 2003).

Nigella sativa L., commonly known as black seed, belongs to the botanical family *Ranunculaceae*. It has been used in many Middle Eastern countries as a natural remedy (Swamy and Tan, 2000). The actual importance of *Nigella sativa* to the Muslims came from the holy saying of the prophet Mohammed prayers and peace be upon him: In the black seed is the medicine for every disease except death (Al-Bukhari, 1983).

Nigella sativa seeds contain 36-38% fixed oils, proteins, alkaloids, saponin and 0.4- 2.5%-essential oil (Ali and Blunden, 2003). Black seed components display a remarkable array of biochemical, immunological and pharmacological actions (Agarwal *et al*., 1979); Boulos 1983; Al-Hader *et al*., 1993; Houghton *et al*., 1995; Haq *et al*., 1999).

Therefore, the aim of the present study was to investigate the possible protective effect of *Nigella sativa* seeds in reducing the biochemical, histopathological and morphometrical changes in liver and kidney of lead acetate-intoxicated rats.

MATERIALS AND METHODS

***Nigella sativa* used:** *Nigella sativa* seeds purchased from the local market, from Cairo, Egypt. The seeds were

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washed, dried in sun and ground before mixing with the basal diet by 5% of the diet weight (Sharaf *et al*., 1996).

Lead acetate used: Lead acetate (Pb $(C_2H_3O_2)_3$) were purchased from Sigma (St. Louis, Mo). The used dose was 10% LD_{50} (466.5 mg kg^{-1} rat b. w.) and 20% LD_{50} (933 mg kg^{-1} rat b. w.) of lead acetate according to Subranamoorthy and Baddaloo (1995) and Nicholas, (1999).

Diets used: The basal diet was formulated from natural ingredients and it consisted of ground corn yellow (60%), wheat flour (2%), Corn glutien (2%), wheat bran (5%), soybean meal (20%), fish meal (0.5%), bone meal (1.5%), ground limestone (1.25%), dicalcium phosphate (0.2%), sodium chloride (2%), mineral premix (3.5%), vitamin premix (1%), methionine (0.2%), lysine (0.5%) and choline bitartrate (0.25%). The basal diet was formulated to meet the rat's nutrient requirements in maintenance as given by The National Research Council (1995) and Reeves *et al*. (1993).

Animals and experimental design: Thirty-six male albino rats of the Sprague-Dawley strains, weighing 90-130 g each, were left under normal healthy conditions at the Animal House of the National Research Centre, Dokki, Cairo, Egypt. Animal were fed on basal diets and water was supplied *ad libitum*. The protocol of this study was approved by the appropriate animal care of the National Research Centre. The animals were divided into six groups each of six rats as follows:

Group 1: Rats fed on basal diet (Control)
Group 2: Rats fed on basal diet plus 5% *Nigella sativa* of diet weight.
Group 3: Rats fed on basal diet plus 10% LD_{50} of lead acetate.
Group 4: Rats fed on basal diet plus 10% LD_{50} of lead acetate and 5% *Nigella sativa* of diet weight.
Group 5: Rats fed on basal diet plus 20% LD_{50} of lead acetate.
Group 6: Rats fed on basal diet plus 20% LD_{50} of lead acetate plus 5% *Nigella sativa* of diet weight.

At the end of the experiment, blood samples were collected after 18 h fasting using the orbital sinus technique of Madway *et al*. (1969). The blood were left to clot in clean dry test tubes and then centrifuged at 3000 rpm for ten min. The clear supernatant serum was frozen at -20°C for the biochemical analysis.

Biochemical methods: Serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities were measured according to the method of Reitman and Frankel (1957). Serum total protein and albumin were estimated according to the methods described by Henry (1964), and Webster (1974) respectively. Serum globulin was calculated according to Latner (1975). Serum urea was carried out according to Patton and Crouch (1977). Serum creatinine was determined by the method of Henry (1974). The methods of Thomas (1992) and Fossati and Principe (1982), respectively, were used to determined serum total cholesterol and triglycerides.

Histopathological study: After blood samples were taken, livers and kidneys were dissected out and fixed in 10% formalin for 24 h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax. Sections of about 6 μm thickness were prepared and then stained with haematoxylin and eosin for histopathological examination (Drury and Willington, 1980). Leica Qwin 500 Image Analyzer in Image Analyzer Unit, Pathology Department, National Research Center was used to measure the damaged areas. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Ten fields were chosen in each specimen and the mean values were obtained.

Statistical analysis: All data were expressed as the mean ± SE. Statistical Analysis was evaluated using the student t-test (Snedcor, 1965). p values less than 0.05 were treated as statistically significant.

RESULTS

Biochemical results: The data in Table 1 demonstrate significant increase in serum level of AST ($p<0.05$) in rats fed on lead acetate with the two levels of doses (10 and 20% of LD_{50}) for six weeks compared to the control group. While significant reductions in AST were recorded in rats fed on lead acetate and *Nigella sativa* as compared with the lead treated groups.

Regarding the changes obtained in serum total protein and albumin, it was found that significant decrease in rats fed on 20% of lead acetate while insignificant changes was recorded when these groups of rates fed on lead plus *Nigella sativa* when comparing with control group. Serum ALT enzyme and globulin fraction insignificantly changed in all groups of rats fed on lead with or without *Nigella sativa* for six weeks.

The results obtained in Table 2 showed significant increases ($p<0.05$) in blood urea and serum creatinine of rats fed on lead acetate with the two levels of doses for six

Table 1: Changes in the levels of AST, ALT, total protein, albumin and globulin in the serum of control, lead treated and lead treated rats given *Nigella sativa*

	Parameters				
Groups	AST (μL^{-1})	ALT (μL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})
Control	5.13±0.27	13.00±1.30	5.70±0.07	4.10±0.02	1.60±0.09
Nigella sativa	6.33±0.42	13.50±0.60	5.50±0.05	4.40±0.07	1.20±0.07
10% LD_{50} lead acetate	10.97±0.42[a]	15.20±1.30	5.20±0.10	4.10±0.10	1.10±0.20
10% LD_{50} lead acetate + *Nigella sativa*	6.37±0.20[b]	13.00±1.20	5.80±0.10	4.20±0.20	1.50±0.30
20% LD_{50} lead acetate	25.67±2.1[a]	14.40±3.20	4.80±0.10[a]	3.70±0.08[a]	1.10±0.20
20% LD_{50} lead acetate+ *Nigella sativa*	11.18±0.26[ab]	11.90±1.10	5.70±0.01[b]	4.50±0.20[b]	1.20±0.20

All data represent as the mean±Standard Error. [a]Significant difference as compared to control group ($p<0.05$). [b]Significant difference as compared to each Pb toxicity group ($p<0.05$)

Table 2: Changes of urea and creatinine of rats of control, lead treated and lead treated rats given *Nigella sativa*

	Parameters	
Groups	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Control	31.10±0.07	0.82±0.01
Nigella sativa	32.30±0.08	0.87±0.05
10% LD_{50} lead acetate	39.80±0.07[a]	1.80±0.02[a]
10% LD_{50} lead acetate and *Nigella sativa*	32.20±0.08[b]	0.93±0.08[b]
20% LD_{50} lead acetate	39.20±0.20[a]	2.00±1.20[a]
20% LD_{50} lead acetate and *Nigella sativa*	31.60±0.05[b]	0.90±0.01[b]

All data represent as the mean±Standard Error. [a]Significant difference as compared to control group ($p<0.05$), [b]Significant difference as compared to each Pb toxicity group ($p<0.05$)

Table 3: Serum total cholesterol and triglycerides of rats fed on lead acetate with or without *Nigella sativa* for six weeks

	Parameters	
Groups	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})
Control	85.50±1.20	42.00±1.20
Nigella sativa	77.60±1.20[a]	46.00±1.20
10% LD_{50} lead acetate	94.00±1.20[a]	74.10±2.40[a]
10% LD_{50} lead acetate and *Nigella sativa*	71.00±1.10[ab]	57.00±2.20[ab]
20% LD_{50} lead acetate	84.40±1.10	60.00±2.20[a]
20% LD_{50} lead acetate and *Nigella sativa*	76.20±1.10[ab]	41.00±1.20[ab]

All data represent as the mean±Standard Error. [a]Significant difference as compared to control group ($p<0.05$), [b]Significant difference as compared to each Pb toxicity group ($p<0.05$)

weeks when compared with the control group. While rats treated with lead acetate and *Nigella sativa* insignificantly changed blood urea and serum creatinine when compared with the control group, but when compared with rats fed on lead acetate only showed significant decrease.

Serum total cholesterol was significant decrease at group of rats fed on *Nigella sativa* only in comparing with control group. In addition, serum total cholesterol and triglycerides exhibited significant increases in rats fed on lead acetate only than control group. Significant decreases were recorded in group of rats fed on lead acetate plus *Nigella sativa* group in comparing with those fed on lead acetate only (Table 3).

Histopathological and morphometrical results: The histopathological examination showed normal architecture of the liver of control rats that fed on the basal diet for six weeks (Fig. 1A) and rats fed on the basal diet in addition to *Nigella sativa* powder (Fig. 1B). Examination of liver of rats fed on the basal diet and ingested 10% LD_{50} of lead acetate, showed dilatation and congestion of the veins in the portal tracts. In addition, periportal necrosis of the hepatocytes that surround the portal areas, and the inflammatory infiltration was seen (Fig. 1C). When *Nigella Sativa* added to the diet to the group of rats fed on 10% LD_{50} of lead acetate, the liver revealed normal structure and the binucleated hepatocytes increased (Fig. 1D). Sections of liver tissues from rats given 20% LD_{50} of lead acetate in the diet showed dilatation, congestion of the veins in the portal tracts and inflammatory infiltration (Fig. 1E). A normal structure of liver was observed when rats were fed LD_{50} of lead acetate plus *Nigella Sativa* in the diet (Fig. 1 F).

Image analyzer examinations for damaged areas showed that there were significant decreases in the damaged areas in rats treated with *Nigella sativa* plus lead acetate as compared with lead acetate treated groups (Table 4).

The histopathological examination of kidney sections showed normal architecture as shown in Fig. 2A. Examination of kidney sections of rats fed on the basal diet in addition to *Nigella sativa* powder showed normal structure of both the renal corpuscles and tubules Fig. 2B. In rats fed on the basal diet and ingested 10% LD_{50} of lead acetate, the renal corpuscle showed congestion and hypercellularity. In addition, highly degeneration of the tubules and hemorrhagic areas in the interstitium are present (Fig. 2C). When *Nigella Sativa* added to the diet to the group of rats fed on 10% LD_{50} of lead acetate, the tubules appeared more or less as normal as shown in Fig. 2D. Sections of kidney tissues from rats given 20% LD_{50} of lead acetate in the diet showed inflammatory infiltration beside the congested renal corpuscle, hemorrhagic areas in the in interstitium. In

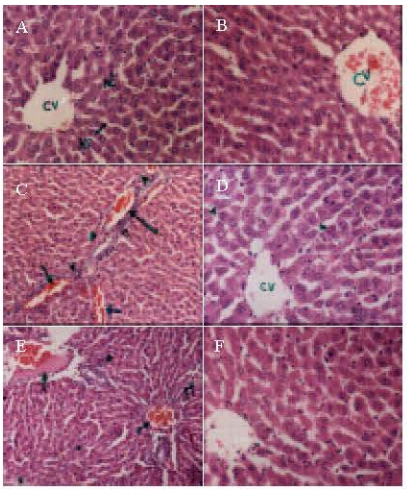

Fig. 1: Photomicrograph of sections in A): Liver of rats of the control shows normal structure. The Central Vein (CV) surround by the Hepato Cytes (HC). Between the strands of hepatocytes the Hepatic Sinusoids are shown (HS), B): liver of rat fed of powder *Nigella sativa* seeds shows normal structure, C): liver of rat fed doses equivalents l0 % of LD^{50} of lead acetate shows a portal tract with dilated and congested veins (arrow). Notice the periportal necrosis of the hepatocytes that surround the portal area (arrowhead) and the inflammatory infiltration (double arrow), D): Liver of rats fed doses equivalents l0% of LD^{50} of lead acetate plus Nigella sativa shows normal structure. Notice the increase of the binucleated hepatocytes (arrowhead), E): liver of rat ingested to 20% of LD^{50} of lead acetate shows a portal tract with dilated and congested veins (arrow). Notice the periportal necrosis of the hepatocytes that surround the portal area (arrowhead), focal necrosis (asterisk), and the inflammatory infiltration (arrows) and F): liver of rats ingested 20% of LD^{50} of lead acetate and Nigella sativa shows the structure more or less as control (H and E stain- X 300)

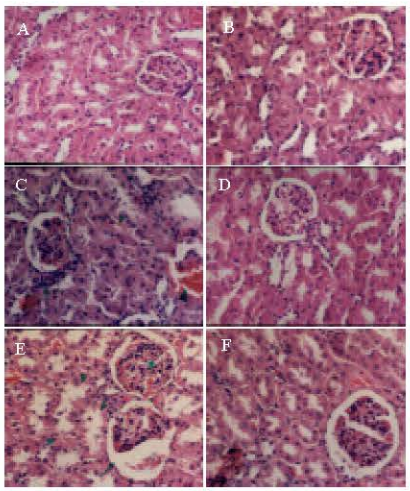

Fig. 2: Photomicrographs of sections in A) and B): Kidneys of control and *Nigella sativa* rat show normal structure, C): Kidney of rat ingested 10% 0f LD^{50} of lead acetate shows congestion and hypercellularity of the renal corpuscle (*) that associated with inflammatory infiltration (arrowhead). Highly degenerative tubules (arrow) and hemorrhagic areas in the interstitium appear, D): Kidney of rat ingested to 10% of LD^{50} of lead acetate plus *Nigella sativa* shows the tubules appear more or less as normal, E): Kidney of rat ingested to 20% of LD^{50} lead acetate shows inflammatory infiltration beside the congested renal corpuscle (*) and patially degenerative one and hemorrhagic areas in the in interstitum (arrowhead). Notice denegation of the renal tubules (arrows). Some tubules show desquamation of its epithelial and F): Kidney of rats ingested to 20% of LD^{50} lead acetate plus *Nigella sativa* shows the renal corpuscles and tubules appear more or less as normal (H and E stain- X 300)

Table 4: Means of damaged areas (μm^2) of liver of rats fed on lead acetate with or without *Nigella sativa* for six weeks

Groups	Area (μm^2)	Area fraction	Area (%)
Control	0	0	0
Nigella sativa	0	0	0
10% LD_{50} lead acetate	595.54±40.13	0.30±0.018	30.00±1.78
10% LD_{50} lead acetate and *Nigella sativa*	249.61±27.59*	0.16±0.02*	16.02±1.72*
20% LD_{50} lead acetate	603.18±40.05	0.35±0.03	35.09±2.56
20% LD_{50} lead acetate and *Nigella sativa*	337.31±25.76*	0.24±0.02*	24.32±1.66*

All data represent as the mean±Standard Error. * Significant difference as compared to each Pb toxicity group ($p<0.05$)

Table 5: Means of damaged areas (μm^2) of kidney of rats fed on lead acetate with or without *Nigella sativa* for six weeks

Groups	Area (μm^2)	Area fraction	Area (%)
Control	0	0	0
Nigella sativa	0	0	0
10% LD_{50} lead acetate	697.03±18.87	0.41±0.01	41. 01±1.84
10% LD_{50} lead acetate and *Nigella sativa*	328.83±26.75*	0.21±0.02*	21.20±1.72*
20% LD_{50} lead acetate	982.08±54.12	0.507±0.03	50.77±2.49
20% LD_{50} lead acetate and *Nigella sativa*	551.15±38.58*	0.31±0.02*	31.01±2.19*

All data represent as the mean±Standard Error. *Significant difference as compared to each Pb toxicity group ($p<0.05$)

addition, denegation of the renal tubules and some tubules show desquamation of its epithelial as shown in Fig. 2E. However, a normal structure of corpuscles renal and tubules were observed when rats were fed 20% LD_{50} of lead acetate plus *Nigella sativa* in the diet (Fig. 2F).

Image analyzer examinations for damaged areas in kidneys indicated that there were significant decreases in the damaged areas in rats treated with lead acetate plus *Nigella sativa* as compared with lead acetate treated group (Table 5).

DISCUSSION

Lead is dispersed thought the environment, in ambient air, in many foods, in drinking water and in dust. The major environmental sources of metallic lead and its salts are paint, auto exhaust, and contaminated food and water (Shalan *et al*., 2005).

The present study was conducted to evaluate the possible protective effect of *Nigella sativa* seeds (Black seed) against the toxicological disorders induced by lead acetate in male albino rats particularly on liver and kidney.

These present results showed that the supplementation of *Nigella sativa* to the diets of rats for six weeks insignificantly changed the biochemical parameters of liver and kidney functions, as well as the histopathological investigations showed normal histological architecture of the liver and kidney. In accordance, the oral administration of the aqueous extracts of *Nigella sativa* seeds showed non significant changes in the hepatocytes of rats (Al-Okbi *et al*., 1997) and in liver or kidney functions (Ali and Blundes, 2003). Another studies also failed to show any toxicity for *Nigella sativa* fixed oil in mice (Zaoui *et al*., 2002; Al Gamdi 2003). In contrast, Tennekoon *et al*., (1991) reported significant elevation of ALT in rats following treatment with aqueous extracts of *Nigella sativa* seeds and there were no significant histopathological changes occur.

This study clearly showed that lead acetate ingestion with concentrations of 10 and 20% of LD_{50} induced a significant elevations of serum AST and significant reduction in serum total protein and albumin after 20% of LD_{50} administration.

Histopathologically, liver showed dilatation and congestion of the veins in the portal tracts, periportal necrosis of the hepatocytes that surround the portal areas, and inflammatory infiltration at the two levels of doses. Othman and El Missiry (1998) has reported that lead treatment exhibited a significant increases in AST, ALT, total proteins, acid and alkaline phosphatases activities, after 3 and 24 h of intramuscular treatment. In accordance with present finding El-Sokkary *et al*. (2005) showed that liver of lead-treated rats revealed remarkable degenerative alterations. lead hepatotoxicity lead to vacuolization of the cells, polymorphism of the nuclei, and a decrease in glycogen content of the hepatocytes (Foulkes, 1996; Pereira *et al*., 2001).

The mechanisms of lead toxicity are poorly understood, but emerging data suggest that some of the effects of lead may be due to its interference with calcium in activation of Protein Kinase C (PKC) and or through production of Reactive Oxygen Spices (ROS). In addition, it was reported that lead increased the level of lipid peroxidation (Upasani *et al*., 2001). Lipid peroxidation leads to lysis and disintegration of many cells as well as alters the mechanical properties of cells (Hochstein and Jain, 1981). Many studies suggest oxidative stress as one of the important mechanisms of toxic effects of lead (Halliwell, 1994; Ercal *et al*., 1996; Gurer *et al*., 1998; Adonaylo and Oteiza, 1999).

Raised serum enzyme levels in intoxicated rats can attributed to the damage structural integrity of the liver (Chenowcth and Hake, 1962), because theses are cytoplasmic in location and are released into circulation after cellular damage (Sallie *et al*., 1991).

In the present study, co-administration of *Nigella sativa* and 10% of LD_{50} lead acetate prevent the elevation of serum AST level but failed to return to the normal level. Serum total protein and albumin exhibited significant increases. Moreover the histopathological examination revealed normal structure and reduction of the damaged areas of the liver of rat's co-administered with lead acetate and *Nigella sativa*.

The protective effect of *Nigella sativa* oil against CCl_4 and D-galactosamine induced hepatic toxicicity in rats was reported by El-Dakhakhny *et al*. (2002) and Al-Gamdi (2003) whom observed reduction in the activities of serum AST, ALT, alkaline phosphatase, lactate and malate dehydrogenases. In addition, other previous studies demonstrated that *Nigella sativa* may be successful in the protection of liver fibrosis in rabbits (Turkdogan *et al*., 2001) and that its oil may play a role against liver damage induced by Schistosoma mansoni infection in mice (Mahmoud *et al*., 2002).

In the kidneys, lead intoxication causes significant increases in blood urea and serum creatinine of rats treated with lead acetate, while the histopathological examination of kidneys revealed congestion and hypercellularity in renal corpuscle. In addition, highly degeneration of the tubules and hemorrhagic areas in the interstitium were present.

Ghorbe *et al*. (2001) illustrated that oral administration of lead acetate caused significant increase in the blood urea and serum creatinine. In parallel, lead intoxication causes interstitial fibrosis, as well as both hyperplasia and gradual atrophy of tubules and glomeruli (Goyer, 1989; Nolan and Shaikh, 1992). Chronic lead exposure caused glomerular and tubulointerstitial changes that associated with glycosuria, proteinuria, renal failure and hypertension (Kim *et al*., 1996; Loghman-Adham, 1997).

The present study showed that the co-administered rats with *Nigella sativa* and lead prevents the elevation of blood urea and serum creatinine and reduction of the damaged ares in kidney tissues. Al-Okbi *et al*. (1997) found that oral administration of *Nigella sativa* seeds and its extracts reveled significant reduction in blood urea nitrogen and serum creatinine.

In the present study, it was found that total cholesterol and triglycerides exhibited significant increases after administration with lead acetate to rats. These results are in agreement with the result of Othman and El Missiry (1998). In addition, rats co-administered *Nigella sativa* to rats intoxicated with lead acetate exhibited significant decreases in total cholesterol and triglycerides levels. Moreover, Serum total cholesterol revealed significant decrease after *Nigella sativa* administered only. These results are in agreement with the previous studies with whole seeds (Sharaf *et al*., 1996; Al-Okbi *et al*., 1997) and/or with oil of *Nigella sativa* seed (Zaoui *et al*., 2002; Badary *et al*., 2002).

The hypocholesterolemia and hypotriglyceridemia of *Nigella sativa* in rats treated with or without lead acetate may be due to the presence of high contents of unsaturated fatty acids (32-40%), moreover, the reduction of serum total cholesterol may be due to the presence of B-sitosterol which is the dominant sterol in *Nigella sativa* (35%) (Muhammad *et al*., 2002).

Patra *et al*. (2001) reported that lead exposure led to increase lipid peroxidation with tissue specific changes in liver, kidneys and brain. However, the lead acetate may induced its toxic effect via peroxidative damage to renal and hepatic cell membrane (Othman and El Missiry, 1998). The preventive activity of *Nigella sativa* may related to its antioxidants efficacy that inhibit lipid peroxidation as well as it appears that may produced pronounced prophylactic action against lead effect. Mansour (2000) indicated that thymoquinone, the major constituent of *Nigella sativa* seeds, is efficient cytoprotective agent against CCl_4-induced hepatotoxicity caused by lipid peroxidation.

In conclusion, the administration of lead acetate led to disturbance in the metabolic pathway in the subjected rats organs especially on liver and kidney, which was affected both biochemically and histopathologically. On the other hand, the co-administered of *Nigella sativa* showed an ameliorated effect on the toxicity of lead acetate and reduction in the damaged areas in liver and kidney.

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