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Possible Protective Effects of Black Seed (*Nigella Sativa*) or Garlic (*Allium Sativum*) Against Lead-Induced Toxicity in growing rabbits

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ABSTRACT

The function of *Nigella sativa* seed (NSS) and garlic has been studied in New Zealand White (NZW) growing rabbits as dietary additions to enhance growth efficiency and detoxification of dietary lead acetate. A total of 90 growing New Zealand White rabbits, 5 weeks old, were divided into six experimental groups randomly, each of 15 rabbits of both sexes. The first and second groups acted as positive and negative indicators, the third and fourth groups got lead acetate plus either *Nigella sativa* seed or garlic. The fifth and sixth groups, respectively, got only *Nigella sativa* seed or garlic.

The data obtained showed that in the negative control group (G2), which provided 0.05 percent lead acetate, the final body weight, the average body weight gain, the productive feed consumed and the performance index of rabbits decreased significantly (P≤0.05). All these traits were slight improved in group 3 and 4, and significantly improved (P≤0.05) in group 5 and 6 of rabbits when compared to the negative control group. Biochemical parameters showed significant increases (P≤ 0.05) in cholesterol levels, triglycerides, aspertate amino transferase (AST), alanine aminotransferase (ALT), urea and creatinine, and significant decreases (P≤ 0.05) in total protein and albumin levels in the negative control group. In groups 3, 4, 5 and 6 of the cholesterol levels of the rabbits, triglycerides, AST, ALT, urea and creatinine decreased significantly (P ≤0.05), as well as, total protein and albumin levels enhanced significantly (P ≤ 0.05) as compared to the negative control treatment. Groups 5 and 6 of rabbits receiving only *Nigella sativa* seed or garlic with basal diet showed no significant variations in the coefficient of digestibility, as well as, in the panel study. On the other hand, lead acetate significantly (P ≤ 0.05) increased the relative weight of the lungs, liver and heart as a proportion of live body weight. Lead acetate exposure significantly increased MDA (biomarker for lipid peroxidation) and decreased GSH and GST and GPx activities. Simultaneously significantly supplemented NSS or dry garlic (P≤ 0.05) reduced MDA concentrations and effectively restored GST, GPx, and GSH output. Additives significantly reduced the accumulation of lead in the liver, kidneys and muscles.

Conclusively from these results it could be concluded that adding 2% *Nigella sativa* seeds or 0.8% dry garlic for that rabbit diet was a safe and realistic approach for reducing lead toxicity in growing rabbit.

1. INTRODUCTION

In Egypt, the concentration of heavy metals in feed exceeded the maximum acceptable limit for fish (Rashed, 2001), vegetables and fruits (Radwan and Salama, 2006), and human organs (Kriegel *et al*., 2006). Lead toxicity is caused by binding essential protein-specific enzymes, hormones, and cell receptors with sulfur groups; removal of certain minerals (calcium, zinc, magnesium, selenium, and copper) from the body; and reduction of carbohydrate, protein, and lipid metabolism (ATSDR, 1993). In addition, the sum of heme synthesis and glutathione had dropped

due to lead (Saxena and Flora, 2004). Lead in liver, kidney and brain has increased lipid peroxidation (Patra *et al*., 2001). Roles of lead compromised adrenal, liver and kidney (Fathi *et al.*, 1999). Garlic has been used to restore the lack of protein enzymes, hormones, cell receptors lost due to lead toxicity in sulfur groups. Garlic protects rats and chickens against lead toxicity (Murugavel *et al.*, 2007). Several studies consider the critical role natural antioxidant plays in prophylaxis of pre-lead toxicity. Garlic has been displaying pharmacological properties and medical applications since ancient times. Notably, garlic contains over 200 medicinal compounds, including allicin, allin, ajone, allinase, peroxidase, S-methyl cysteine and volatile sulfur oil (Block, 1985). Garlic has been used effectively to trigger immune function, improve foreign compound detoxification, antimicrobial and antioxidant activity (Banerjee et al., 2001). Garlic is a good source of nutrition (sulfur, minerals, vitamins, fatty acids, and essential amino acids), boosts glutathione enzymes, prevents carcinogenicity and mutagenicity (Shukla and Kalra, 2007), and enhances immune system and organ function (Shehata *et al*., 2003).

Nigella sativa, a member of the Ranunculaceae family, also known as black seed or black cumin, has a wide range of pharmacological activities including; antidiabetic, carminative, anti-pyretic, analgesic, anti-inflammatory, anti-diarrheal and antibacterial activity, anti-oxidant action, and hepatic damage protection (Lutterodt *et al*., 2010). It has nigellon, thymoquinone and thymohydroquinone, proven to have antimicrobial activity and improve interleukin-3 and 2 beta development by lymphocytes and to have an effect on macrophages (Haq *et al*., 1995). Some studies have shown that the seed of Nigella has immunostimulating effects, maintaining good animal health (Al-Beitawi and El-Ghousein, 2008). *Nigella sativa* seed has over 100 different chemical components including mucilage, sugar reduction, crude fiber, alkaloids, resins, flavonoids, organic acids (sterols, tannins and saponins), in addition to high levels of unsaturated fatty acids, especially oleic acids and linoleic acids and proteins. It also has yellowish volatile (essential) oil (Gilani *et al*., 2004). Its essential oil components are understood to be due to the biological activity of the *Nigella sativa* seeds (Hajhashemi *et al*., 2004).

Therefore, the aim of this study was to expose *Nigella sativa* seeds or garlic's ability to reduce lead toxicity on the production of rising rabbits.

2. MATERIALS AND METHODS

This study was conducted at Sakha Station's Rabbits Farm, Animal Production Research Institute, Agriculture Research Centre, Egypt.

A total of 90 growing New Zealand White rabbits, 5 weeks old, were divided into six experimental groups randomly and equally, each of 15 rabbits of both sexes. The experimental groups were classified as follow:

Group 1: fed the basal diet only and served as positive control. Group 2: fed the basal diet containing 0.05% lead acetate and served as negative control (contaminated diet), Group 3 : fed the contaminated diet plus 2 % *Nigella sativa* seed. Group 4: fed the contaminated diet plus 0.8% dry garlic. Group 5 : fed the basal diet containing 2 % *Nigella sativa* seed. Group 6 : fed the basal diet containing 0.8% dry garlic.

Rabbits were housed with fodder and automatic nipple drinkers in single galvanized wire flat deck batteries (60 x 50 x 35 cm). The batteries were naturally ventilated in the rows of a windowed building. Throughout the experiment, a period of 16 hours of light and 8 hours of dark was used. All the rabbits were housed under the same conditions of care. According to De Blas and Mateos (1998), the experimental diets were developed to cover all critical nutrient requirements for rabbit increasing. The experimental rabbits were kept under the same managerial and hygienic condition. Table (1) demonstrates experimental diet combinations and nutrient composition.

Lead acetate, *Nigella sativa* seed or garlic were added to basal diet ingredients before pelleting. Lead acetate was purchased from the pharmaceutical chemicals company El-Nasr (Mansoura, Egypt) ([$Pb(CH_3CO_2)_2$], 99 per cent pure). *Nigella sativa* seeds from Cairo, Egypt, purchased from the local market. The seeds were washed, dried in sun and soil until 2 percent were combined with the basal diet. Hassan and Alaqil (2014) have shown that black seed contains 20.24% CP, 32.86% EE, 13.93% CF, 6.73% ash and 2490 Kcal / kg digestible energy. Garlic was dried for 72 hours without skin and then ground to become powder. Fresh garlic contains dry matter of 40 percent. Approximate dry matter study, which contained 95.50 percent OM, 11.00 percent CP, 8.25 percent CF, 3.25 percent EE, 73.00 percent NFE 4.5 percent ash and 2673 Kcal / kg digestible energy (Shehata, 2011). Other studies have been based on the doses of lead acetate, *Nigella sativa* seed and garlic (Yassin *et al*., 2005; Shehata, 2011 and El-Far *et al*. 2017).

Individual body weight and intake of feed have been reported weekly from 5 to 13 weeks of age. Both assessed were BWG and feed conversion ratio. The weight gain (BWG) was calculated by subtracting BW at beginning of period and at the end of the period. Feed intake (FI) was calculated as the difference between the weight of the feed offered and the weight of the remained at the same day of weighing the animals. Feed conversion ratio (FCR) was computed as the ratio between g FI and g weight gain per period.

Performance index (PI) = (Final live body weight (kg)/ Feed conversion ratio) × 100 (North 198).

At the conclusion of these tests, slaughtered rabbits (6 rabbits / treatment), determined carcass characteristics, obtained blood samples to investigate carcass and blood parameters. Some carcass characters were relative weights of dressing and some organs. Using commercial kits obtained from Diamond Diognastics Company, Egypt, serum albumin, total protein, alanine amino transaminase (ALT), aspertate amino transaminase (AST), creatinine, urea, triglycerides and cholesterol were analyzed. Subtracting the albumin values from the corresponding total protein values calculated the globulin values. An atomic absorption spectrophotometer (model 210 VGP, Buck Scientific, USA) with an oxidizing air acetyleneflame was used to assess the residue of lead in the kidneys, muscles and liver of 6 rabbits of each procedure. According to A.O.A.C. (2000), a similar study of feed and feces was determined. All the experimental diets were formulated to be iso-nitrogenous, iso-caloric, and to meet all the essential nutrient requirement of growing rabbits according to Lebas (2013) as shown in Table 1. Feed consumption and excreted feces of 6 rabbits per treatment were reported daily for digestibility tests during the last week of the experiment. Digestibility coefficients of nutrients (DM= Dry matter, OM=Organic matter, CP= Crud protein, EE= Ether extract, CF= Crud fiber, NFE= Nitrogen free extract) and nutritive values (DCP= Digestibility crud protein, TDN= Total digestibility nutrient) were determined.

2.1. Preparation of liver tissue homogenate

The test rabbits and study groups (n=6) were sacrificed, then dissected liver samples and dissolved in ice-cold saline by 0.9 per cent. Samples were homogenized in 0.1 M Tris-HCl pH 7.4 buffer containing 5 mMβ-mercaptoethanol (1:4 w / v), using a motor-driven Teflon and glass Potter-Elvehjem homogenizer. At 4° C, the homogenates were centrifuged at 105,000 x g for 60 min; the supernatants were divided into aliquots and then processed for further oxidative stress assessment at -20 ° C.

2.2. Determination of oxidative stress parameters

The homogeneous frozen liver aliquots were used for colorimetric evaluation of the MDA and GSH content, as well as the function of the enzyme GST and GPx.

2.4. Determination of lipid peroxidation

The principal aldehyde by-product of lipid peroxidation in biological systems is malondialdehyde (MDA). It was analyzed to form reactive thiobarbituric acid (TBARS), a pink colored compound, following the incubation of supernatants with thiobarbituric acid at 95 ° C for 30 min (pH 3.6). MDA levels, measured at 532 nm, were expressed as nmol MDA / mg protein (Ohkawa *et al.*, 1979).

2.5. Determination of reduced glutathione (GSH) levels

Reduced glutathione assay was based on DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] cleavage reduction by compounds containing sulfhydryl groups and yellow color production (Sedlak and Lindsay 1968). The decreased chromogenic content is directly proportional to the degree of GSH. The absorbance in μmol GSH / mg was measured to be 412 nm and expressed as protein.

2.6. Determination of the glutathione S-transferase (GST) activity

GST operations were tested using the methods of Vessey and Boyer methods (1984). This assay was focused on monitoring the CDNB [1-chloro-2,4-dinitrobenzene] enzyme-catalyzed conjugation rate with GSH. GST activity was calculated as 340 nm increases in absorbance, and as CDNB / min / mg protein l mol.

2.7. Determination of the glutathione peroxidase (GPx) activity

The operation of GPx was analysed using the methods of Chiu *et al*. (1976) method. The assay is focused on measuring oxidized glutathione (GSSG), which is produced by reducing organic peroxide in the GSH, GR, NADPH, and Tris–HCl reaction mixture. In order to increase absorption by 340 nm, GPx activity was measured and identified as GPx units / mg protein.

Table (1). Composition and chemical analysis of the experimental diets

Ingredients (%)	Experimental diets					
	Basal Diet	0.05% lead acetate	0.05% lead acetate +2% *Nigella sativa*	0.05% lead acetate + (0.8% dry garlic)	2 % *Nigella sativa* seed	2% fresh garlic (0.8% dry garlic)
Yellow corn	17.0	17.0	17.0	17.0	17.0	17.0
Clover hay	30.7	31.2	30.7	31.2	30.3	31.0
Wheat bran	18.0	15.15	13.9	15.15	16.5	16.3
Barley	10.0	11.7	11.5	12.0	10.1	10.0
Soybean meal (44%)	18.0	18.6	18.1	18.6	17.7	18.5
Molasses	3.0	3.0	3.0	3.0	3.0	3.0
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3
Primex[1]	0.3	0.3	0.3	0.3	0.3	0.3
Di-calcium phosphate	1.7	1.7	1.7	1.7	1.7	1.8
Limestone	0.8	0.8	0.8	0.8	0.9	0.8
Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Lead acetate	0.0	0.05	0.05	0.05	0.0	0.0
Nigella sativa seed	0.0	0.0	2.0	0.0	2.0	0.0
Dry garlic	0.0	0.0	0.0	0.8	0.0	0.8
Total	100	100	100	100	100	100
Chemical composition[2](% of DM basis)						
Crude protein	17.02	17.09	17.1	17.09	17.03	17.03
Crude fiber	13.00	13.00	13.09	13.00	13.00	13.03
Ether Extract	1.80	1.75	2.34	1.75	2.40	1.78
Ash	5.61	5.56	5.57	5.57	5.61	5.62
NFE	62.57	62.68	62.07	62.69	62.00	62.54
Calcium	1.21	1.21	1.21	1.22	1.24	1.23
Phosphorus	0.780	0.755	0.748	0.755	0.761	0.784
DE (kcal/kg)	2434	2444	2444	2444	2434	2435
Lysine	0.840	0.845	0.824	0.853	0.821	0.845
Methionine	0.454	0.453	0.447	0.453	0.448	0.453

[1]Vit+Min mixture provides per kilogram contains: Vit A 6000 IU; Vit D3 450 IU; Vit E 40 mg; Vit K3 1 mg; Vit B1 1 mg; Vit B2 3 mg; Vit B3 180 mg; Vit B6 39 mg; Vit B12 2.5 mg; Pantothenic acid 10 mg; biotin 10 mg; folic acid 2.5 mg; choline chloride 1200 mg; Manganese 15 mg; Zinc 35 mg; Iron 38 mg; Copper 5 mg; Selenium 0.1 mg; Iodine 0.2 mg; Selenium 0.05 mg.
[2]Calculated according to De Blas and Mateos (1998) and NRC (1977).

2.8. Determination of tissue Protein

The Bradford (1976) method used bovine serum albumin as the standard for determining protein concentrations in the homogeneous tissue.

2.9. Statistical analysis

All data were analyzed using one-way variance analysis (ANOVA) using statistical software SPSS 11.0 (SPSS, 2001). The new multi-range Duncan test (Duncan, 1955) was used for the detection of significant differences ($P \leq 0.05$) between methods.

3. RESULTS AND DISCUSSION

3.1. Rabbit performance

The effects on growth efficiency of the experimental diets are presented in Table 2. Results showed that rabbit that fed on 2 percent of *Nigella sativa* seed (NSS) or 0.8 percent of dray garlic significantly enhancement of the final body weight, total weight gain and daily weight gain by (0.76 and 1.55 percent), (1.42 and 2.43 percent) and (1.43 and 2.45 percent) feed conversion ratio by 3.24 and 4.42 percent, and performance index by 4.33 and 6.38 percent, respectively, compared to control. The final body weight, total weight gain, daily weight gain, average daily feed consumption, feed conversion and performance index that presented in Table (2) decreased significantly (13.67, 20.60, 20.59, 5.61, 19.17 and 27.35%) relative to the stable control when adding lead acetate. Reducing the intake of feed was accepted with those received by ATSDR (1993) who confirmed that exposure to lead induced loss of appetite. Nevertheless, the reduction of BWG was in accordance with Falke and Zwennis (1990) who found that the addition of lead acetate (0.80-1.20 µg / kg body weight for three days / week) reduced the weight of the female rabbit. In fact, wistar rats, exposure to 1 % or 0.7 % lead acetate, decreased body weight in their diet (Walsh and Ryden, 1984). Diminishing feed intake

and regular body weight gain can be attributed to adverse health effects of lead (irritability, metallic taste, headache, muscle and joint pain, colic, abdominal pain, constipation, nausea, cramps, nervous disorder, vomiting, hematopoietic and cardiovascular system and kidney and liver function); reduces of certain minerals (Zn, Ca, Se, Mg and Cu) from the body (ATSDR, 1993); reduced digestibility of nutrients (Fekete et al., 2001), and increased hormones T3 and T4 (Fathi *et al.*, 1999).

Addition of *Nigella sativa* seed or garlic (Table 2) significantly improve growth efficiency ($P \leq 0.05$) compared to those diet-fed lead alone. The improvement in the average daily body weight gain, respectively 22.64 and 23.16 %, with 2 percent *Nigella sativa* seed and 0.8 percent dry garlic content. Because of its content, the beneficial effect of garlic may be due to increased feed consumption and nutritional values, decreased the bonds of lead by sulfur groups on enzyme protein, hormones and cell receptors by enhancing the excretion of lead in fecal and urine (Flora and Seth, 1999), stimulating the immune system and organ work by using garlic (Murugavel *et al*., 2007). However, lead acetate had an adverse effect on feed consumption (Table 2). Such findings were consistent with Fathi *et al*. (1999) who found that broiler chicks feed 200-400 ppm lead chloride resulted in poor conversion of feed. The application of *Nigella sativa* or garlic significantly improved feed conversion. Improvement of feed conversion may be due to improved feed consumption capacity, reduced animal discomfort, and improved functioning of organs for growing rabbits fed diets of *Nigella sativa* or garlic powder.

Because of its antioxidant properties and phenolic compounds, part of the increase in the growth of the rabbits obtained herein could be due to the positive impact of *Nigella sativa* on body weight gain and feed conversion ratio. Yasser *et al*. (2015) recorded that at the end of the experiment, the highest body weight values (body weight gain and economic efficiency for group of rabbits fed *Nigella sativa* seed (0.4%) were observed compared to the control group. AbuAl-Basal (2011) documented the diversifying ability of the active substances Nigellone and Melation and worked synergistically to enhance the use of nutrients and to help eliminate and clean up behavior. Moreover, Nigellone had both anti-spasmodic and bronchodilating effects that could improve the health of the respiratory tract, which is a major problem under hot conditions. Nevertheless, because of its anti-inflammatory, analgesic, antioxidant and cleaning effects, thymoquinone helps the body to get rid of toxins (Paarakh, 2010). Al-Douri *et al*. (2010) also stated that the seed of *Nigella sativa* had an antimicrobial effect that may help rabbits get started and have a hot climate. *Nigella sativa* also contains non-identified fat-soluble factors and a mixture of essential fatty acids, including linolenic, linoleic, and arachidonic acids, which were important growth factors (Murray *et al*., 1991) and/or macro-elements such as Ca, P and Mg, which are required for optimal bone formation and growth throughout the growing period. *Nigella sativa* seeds also possessed many vital microelements that were known as growth elements and played an important role in this phenomenon, such as Cu, Zn and Fe (William, 1999) and essential vitamins (thiamine, riboflavin, pyridoxin, niacin and folacin) that played a role in improving growth through their effects on the metabolism of fats, proteins and minerals (McDowell, 1989).

3.2. Mortality rate

During the experimental phase, the percentage of mortality rate was not affected in all groups studied.

3.3. Digestibility and nutritive values:

The digestibility of most nutrients and nutrient levels decreased significantly (Table 3). These results were an agreement with Bersenyi *et al*. (1999) who found that when carrot-fed rabbits consumed high levels of lead, the digestibility of crude protein (CP) decreased. However, in potato-fed rabbits and high-lead beetroot, Fekete *et al*. (2001) found reduction in digestibility of organic matter (OM) and nitrogen-free extract (NFE). Reducing the digestibility of lead nutrients can be due to protein, carbohydrate and lipid metabolism disruption, copper and manganese displacement (both require optimal adrenal function) (ATSDR, 1993), reduction of T3 hormones and liver function (Fathi *et al*., 1999), increased lipid peroxidation that suggests oxidative damage to organs (liver, brain and kidney) (Patra *et al.*, 2001), and the destruction of useful bacteria (this may be the reason why CF digestibility is decreased). Supplementation of *Nigella sativa* or garlic significantly increased as a total digestible nutrient (TDN percent) and digestible crude protein (DCP percent) DM, OM, CP and CF digestibility and nutritional values. These findings are in accordance with Yasser *et al*. (2015) who found the apparent digestibility of nearly nutrients OM, CP, CF, EE and NFE was significantly increased with black cumin compared to the control diet. These results may be due to restoring essential protein, which plays an

important role in nutrient indigestion, increasing glutathione enzymes in the liver, protecting cells from oxidative damage, and playing a beneficial role in detoxification, inhibiting lipid peroxidation, improving organ function, and improving immunity (Patra et al., 2001 and Shehata *et al*., 2003). Jamroz and Kamel (2002) reported a stimulating effect on the digestive system of black seed, caused to better absorption and hence improved performance. Because the inclusion of *Nigella sativa* in feed enhances the bile flow rate, this effect caused to enhanced emulsification that activates the lipases pancreatic and then aids in digestion of fat and absorption of fat-soluble vitamins.

3.4. Blood parameters:

The goal of this study was to evaluate the potential preventative effect of *Nigella sativa* seeds (Black Seed) and garlic on toxicological disorders caused by lead acetate in rabbits, especially in the kidney and liver.

The findings in this study indicate that addition of *Nigella sativa* seeds or garlic powder to eight weeks of rabbit diets has modified the biochemical parameters of liver and kidney functions in an insignificant way. Accordingly, there was no significant improvement in rat hepatocyte oral administration of *Nigella sativa* aqueous extracts (Al-Okbi *et al.,* 1997), and liver or kidney functions (Ali and Blundes, 2003). However, another study showed no toxicity at mice to fixed Nigella sativa oil (Al Gamdi 2003).

As shown in Table (4), only in comparison to control treatment, serum total protein, albumin and globulin were significant enhances in rabbit groups fed diets containing *Nigella sativa* or garlic. Hassan and Abotaleb (2007) reported the same results; they observed an increase in serum albumin. The levels of albumin are taken as measures of liver synthetic activity (Black, 1996). A common mechanism appears to affect albumin resulting in a response that could be a *Nigella sativa* induction of enzymes (AlJishi and Abuo, 2003).

As a result of lead toxicity, the serum total protein, globulin and albumin levels were significantly reduced ($P \leq 0.05$). These results comply with Fathi et al's stated findings (1999) the lead chloride has been reported to decrease total protein in broiler chick serum. Falke and Zwennis (1990) also stated that lead in female rabbits caused anemia (lead inhibiting main enzymes involved in heme synthesis). Serum protein decrease may be due to the inhibition of lead-induced protein synthesis. Since lead enhances the degradation of essential proteins and the activity of erythrocyticdelta-aminolaevulinincdehydratase (ALA-D) (an important enzyme in the synthesis pathway). Glutathione levels were also significantly decreased by addition of lead, which reduces liver function (Saxena and Flora, 2004). Declining globulin levels per lead can suggest an immunodepressive response (Shehata *et al*., 2003). Lead acetate has greatly improved the function of the AST and ALT enzymes (Table 4). Toxic hepatosis can cause AST activity to rise (Abd El-Hamid and Dorra, 1993). Regarding control, the creatinine levels in the serum of rabbits fed the lead diet were higher. These results were consistent with Fathi *et al.* (1999) who said that lead had a negative effect on the histopathology of the kidneys and decreased their function. The enhancement in serum urea and creatinine may be due to inhibition of kidney excretion or increased liver synthesis from ammonia. The rise in creatinine is also associated with increases in muscle atrophy (Abd El-Hamid and Dorra, 1993). Enhanced catabolism of proteins and accelerated gluconeogensis dominance of graded amino acids is likely and appropriate to interpret the elevated urea level. On the other hand, during lead administration, the elevated serum urea levels may be due to the degradation of red cells. Some toxic compounds can increase blood urea and decrease the protein of plasma (Varely, 1976). Data from Table 4 showed that the addition of *Nigella sativa* or garlic improved all tested serum parameters, respectively. These findings are consistent with those obtained by Abdel-Razik *et al.* (2007) who reported co-administration of *Nigella sativa* and 10 per cent of LD_{50} lead acetate to prevent serum AST levels from being elevated but have failed to return to normal. Total protein and serum albumin have seen significant increases. Additionally, the histathological examination showed normal structure and reduction of impaired areas of rat liver co-administration with lead acetate and *Nigella sativa*. Al-Gamdi (2003) reported the protective effect of Nigella sativa oil on D-galactosamine mediated hepatic toxicity in rats, which observed a reduction in the activities of serum AST, ALT, alkaline phosphatase, lactate, and malate dehydrogenases. Nonetheless, other earlier studies have shown that *Nigella sativa* can be effective in preventing liver fibrosis in rabbits (Turkdogen *et al*., 2001), and that its oil can play a role in mice against liver damage to Schistosomamansoni (Mahmoud *et al.*, 2002).

Table (2). Effects of *Nigella sativa* seeds (NSS) and garlic on productive performance of growing rabbits from 5 to 13 weeks of age.

Items	Dietary treatments						SEM	p-value
	Control	Lead acetate	lead+ NSS	lead+ garlic	N. sativa	garlic		
Initial weight (g)	777.8	775.1	776.2	777.0	773.8	776.6	0.53	0.284
Final weight (g)	2273.2^{c}	1962.3^{e}	2232.3^{d}	2239.2^{d}	2290.5^{b}	2308.6^{a}	12.56	0.0001
Total weight gain (g)	1495.3^{c}	1187.2^{e}	1456.0^{d}	1462.2^{d}	1516.7^{b}	1532.0^{a}	12.53	0.0001
Daily gain (g)	26.70^{c}	21.20^{e}	26.00^{d}	26.11^{d}	27.08^{b}	27.35^{a}	0.22	0.0001
Daily feed intake (g)	90.74^{a}	85.65^{c}	91.32^{a}	88.51^{b}	88.89^{b}	88.76^{b}	0.22	0.0001
Feed conversion ratio	3.39^{c}	4.04^{a}	3.51^{b}	3.39^{c}	3.28^{d}	3.24^{d}	0.02	0.0001
Performance index (%)	66.90^{c}	48.60^{e}	63.56^{d}	66.06^{c}	69.80^{b}	71.17^{a}	0.80	0.0001

$^{a, b, :}$ Values in the same row with different superscripts differ significantly (P≤ 0.05)

Table (3). Effect of *Nigilla sativa* seeds (NSS) and garlic on digestibility coefficients of nutrients and nutritive values

Items	Dietary treatments						SEM	p-value
	Control	Lead acetate	lead+ N. sativa	lead+ garlic	N. sativa	Garlic		
Digestion coefficient (%):								
DM (%)	64.59^{a}	60.61^{c}	64.30^{a}	63.50^{b}	64.71^{a}	64.45^{a}	0.251	0.0001
OM (%)	57.71^{a}	54.66^{b}	57.51^{a}	57.75^{a}	57.71^{a}	57.26^{a}	0.210	0.0001
CP (%)	74.18^{b}	70.85^{c}	73.85^{b}	73.63^{b}	75.03^{a}	75.05^{a}	0.253	0.0001
EE (%)	62.67^{a}	59.95^{c}	61.49^{b}	60.93^{b}	63.01^{a}	63.16^{a}	0.224	0.0001
CF (%)	31.47^{bc}	29.98^{d}	31.09^{c}	31.60^{abc}	32.05^{ab}	32.25^{a}	0.152	0.0001
NFE (%)	51.72^{a}	50.00^{c}	51.13^{b}	51.16^{b}	51.73^{a}	51.70^{a}	0.119	0.0001
Nutritive value (%):								
TDN (%)	51.62^{b}	49.60^{d}	51.64^{b}	51.09^{c}	51.83^{b}	52.39^{a}	0.153	0.0001
DCP (%)	12.62b	12.07^{c}	12.57^{b}	12.53^{b}	12.77^{a}	12.76^{a}	0.042	0.0001

$^{a, b,}$: Values in the same row with different superscripts differ significantly (P≤ 0.05); DM= Dry matter, OM= Organic matter, CP= Crud protein, EE=Ether extract. CF=Crud fiber, NFE=Nitrogen free extract,DCP= Digestibility crud protein, TDN=Total digestibility nutrient.

Total cholesterol significantly decreased only in comparison with control group in rabbit groups fed on *Nigella sativa* or garlic. Results are in line with those AL-Beitawi *et al*. (2009) reporting that N. Sativa significantly reduced total cholesterol and triglyceride serum levels. The decline in plasma cholesterol levels can be due to Nigella sativa's high content of unsaturated fatty acids that can induce cholesterol excretion in the intestine and oxidation (Khodary *et al*., 1996). However, serum triglycerides and total cholesterol showed significant rises in rabbits fed only on lead acetate than control group. The increase in cholesterol content was agreed with that recorded by Ashour (2002) in response to 20-day lead acetate feeding in rabbits. In treatments of rabbits exposed to lead acetate with *Nigella sativa* or garlic groups, substantial reductions were observed compared to those feeding on lead acetate only (Table, 4). Patra *et al*. (2001) also confirmed that *Nigella sativa* prevents the oxidative damage caused by lead in liver, kidney and brain. Garlic powder had some components that could activate the immune system and the role of blood cell-related organs such as bone marrow, spleen and thymus (Ali *et al*., 2000).

3.5. Antioxidants parameters and lipid peroxidation:

Our inquiry investigated whether dietary supplementation with *Nigella sativa* and garlic avoids or even mitigates the hepatic oxidative harm caused by exposure to lead. The rabbits initially showed a normal behavior with no evident signs of toxicity and a survival rate of 100 percent over the entire duration of the study compared with the control treatment. Table 5 demonstrates the hepatic oxidative effects of lead and the antioxidant effectiveness of *Nigella sativa* or garlic. It was noted that *Nigella sativa* and garlic supplementation evoked a substantial ($p \leq 0.05$) depletion of MDA (4.01 or 4.13 nmol / mg protein) respectively, and an improvement in GSH content (69.00 or 70.70 mmol / mg protein) relative to control values respectively. Although GPx and GST activities

were comparable with each other in the management of hepatic tissues and *Nigella sativa* and garlic supplemented rabbits. In comparison, lead consumption was significantly ($p \leq 0.05$) increased lipid peroxidation bio-indicator, MDA (39.30nmol / mg protein), and significantly reduced ($p \leq 0.05$) GSH content (33.36mmol / mg protein), GST activity (28.11U / mg protein) and GPx activity (6.45 U / mg protein) in the liver of rabbit. In addition, the oxidative effects of lead were reduced by *Nigella sativa* and garlic. This caused reduction of MDA (protein 19.52 and 18.48nmol / mg) and induction of GSH (protein 45.60 and 48.06mmol / mg) and GST (protein 34.95 and 35.25 U / mg) and GPx (protein 10.77 and 11.00U / mg) behaviors that remained comparable to control group.

The antioxidant system includes enzymatic (CAT, GPx,, SOD, GST, and GR) and non-enzymatic (vitamin E, vitamin C, GSH, phenolic and metallothionine) antioxidant molecules (El-Far *et al*., 2014, El-Sayed *et al.*, 2015) defensive pathways. An impaired antioxidant function can cause damage to the cell membrane, changes in the fluidity and permeability of the membrane, and oxidative stress (Lu, 2009). Oxidative stress results from a deficiency in the antioxidant protection and the accumulation of ROS result in oxidative damage to important cell biomolecules such as DNA, lipids and proteins (Halliwell and Gutteridge 2007). Lead exposure at a concentration of 100 mg / kg diet significantly increased MDA levels and decreased GSH content, as well as GST and GPx enzyme activities, reflecting the degree of Pb-induced oxidative stress and ROS production. Rat sensitivity to lead has been reported to result in significant decreasing in SOD, CAT and GPx activities in addition to a significant enhance in MDA (Mohamed *et al.*, 2016). The same authors reported that there was a negative relationship between lead levels and GSH content and regeneration rate, as well as activity on GPx and GST enzymes. The pathways misinterpreting the oxidative damage caused by Pb. However, the effects of lead may result in interference with calcium inactivation of protein kinase C (Upasani *et al*., 2001) and/or 5-aminolevulinic acid dehydratase (ALAD) in ALA (Lalith Kumar, 2014) and there by ROS. Lead induced oxidative tissue damage could be mediated as a result of reduced antioxidant function, enhanced production of ROS or both, which suggested enhanced lipid peroxidation. Additionally, Pb was found not only to inhibit the activity of GPx enzymes, but also to cause a drastic decline in protein expression, thereby depleting hepatocellular defense against oxidative stress. Hence, blocking or retarding oxidation production could be promising strategies for preventing oxidative hepatic harm. In many studies there is an inverse correlation between dietary antioxidants and oxidant damage (El Far *et al*., 2016). Dietary supplementation of natural antioxidants (*Nigella sativa* and garlic) is considered to be a type of preventive medicine; therefore, it is important to research natural sources of antioxidants.

The preventive function of *Nigella sativa* may be due to its antioxidant effectiveness, which both prevents lipid peroxidation and appears to have established pronounced prophylactic activity against the effect of lead. Mansour (2000) indicated that thymoquinone is an effective cytoprotective agent against lipid peroxidation-induced hepatotoxicity caused by CCI4, the major component of *Nigella sativa* seeds.

Here, it was observed that dietary supplementation of *Nigella sativa* substantially inhibited lipoperoxidation (LPO) in Pb-intoxicated rabbits. However, the current study showed that dietary supplementation of *Nigella sativa* significantly improved GSH efficiency and activity of GSH-dependent enzymes, GPx, and GST. This improved the GPx and GST detoxifying scavenging ROS and metabolic peroxides and hydroperoxides (Krishnan and Muthukrishnan, 2012). In particular, it was noted that supplementation with *Nigella sativa* significantly increased the expression of the hepatic protein Se-GPx which could be due to the activation of its hepatocellular biosynthesis. Animals treated with *Nigella sativa* have shown substantial decreases in hepatic lipid peroxidative marker levels with co-improvement in the antioxidant system (Abdel-Daim and Ghazy, 2015). Nigella sativa therefore has significant antioxidant and free radical scavenging properties that could be attributed to its major component of thymoquinone, carvacrol, 4-terpineol, dithymoqui- none, and thymol that protects against oxidative hepatic damage (Ijaz, *et al*., 2017). All in all, these results suggested that Pb-induced LPO is partly due to disruption of the GSH and GSH-dependent enzyme homeostasis, and their defense is one of Nigella sativa's possible mechanisms to defend against Pb-induced hepatic oxidant stress.

The results show that garlic may have contained chelating compounds capable of enhancing lead (Pb) removal. Garlic feeding can be used to limit human consumption by reducing the levels of lead (Pb)

in food animal bones (Hanafy *et al*., 1994). Ail's ability to provide glutathione, biosynthesize metallothionine, or related protein, and its antioxidant properties tend to protect against possible lead oxidative damage (Pb) (Tandon *et al*., 2001). This finding is consistent with several scholars documenting the analysis (Bakalli *et al*., 1995). In addition to chelation, other garlic compounds (Sallylcystine, S-allylmercaptocysteine and some micronutrients) also prohibit the gastrointestinal tract from consuming lead (Pb) (Pourjafar *et al*., 1996). Garlic also includes several compounds of oil-soluble organosulfur, flavonoids, phenol allixin, and other beneficial nutrients, including selenium. (Amagase *et al*., 2001). It also enhances cellular antioxidants, including glutathione, that help maintain a healthy immune system and prevent drug toxicity, and peroxidases that remove toxic peroxides (Wei *et al*., 1998).

3.6. Some carcass traits:

The level of dressing in rabbits treated with lead was significantly lower (P≤ 0.05) relative to lead-plus additives treated, while the liver, kidneys and heart weight decreased as a percentage of live body weight increased (P≤ 0.05) with the introduction of lead acetate (Table 6). Such findings were accepted with Fathi *et al*. (1999) who stated that 200-400 ppm lead chloride in broiler diets significantly reduced (P≤05%) the dressing percentage and increased the heart rate as a percentage of live body weight. Such significant changes in organ weights may be due to swollen mitochondria in these organs ' proximal tubule cells, or may be due to subsequent cell damage, hepatic fibrosis, and nucleus position of lead could be identified as the most ultra-structural characteristics of lead poisoning diagnosis. Birkhead *et al*. (1982) also obtained similar results. Improvements have been observed in carcass characteristics in lead-treated rabbits plus *Nigella sativa* or garlic compared to the infected diet group (Table 6). Such findings can be due to NSS or garlic promotional action that can provide adequate protection from lead toxicity. Shehata, (2011) found that the amount of dressing in rabbits fed diet contaminated with lead acetate only decreased significantly compared to fed lead plus additives (0.08%, 0.16% DL-methionine and 2% fresh garlic) and power. On the other hand, lead acetate significantly increased the weight of the heart and kidneys as a proportion of the live body weight.

At the end of the experimental phase, the flavor test performed by 10 people on cooked meat found that there were no variations between the different treatments.

3.7. Residue of lead:

Experiments were carried out to determine the capacity for dietary *Nigella sativa* or garlic to affect the residual deposition of lead (Pb) in rabbits caused by lead toxicity in liver, kidney and muscles. Table 6 shows the residual deposition of lead (Pb) in the control and experimental animal groups of the liver, kidney and muscle tissues. *Nigella sativa* or garlic (*Allium Sativum*) were also investigated for its ability to avoid lead (Pb) accumulation in order to reduce the residual deposition of lead (Pb) in the liver, kidney and rabbit muscles. The concomitant use of garlic supplement and lead acetate in rabbits has been found to reduce the tissue (Pb) lead load, suggesting substantially the possible therapeutic effect of garlic against lead toxicity, which is very close to the previously reported rat findings (Senapati et al., 2001).

Table (4). Effects of *Nigella sativa* seeds (NSS) or garlic on some blood parameters

Items	Dietary treatments						SEM	p-value
	Control	Lead Acetate	lead+ N. sativa	lead+ garlic	N. sativa	Garlic		
Total protein (g/dL)	6.61^{a}	4.91^{d}	6.06^{c}	6.31^{b}	6.60^{a}	6.58^{a}	0.104	0.0001
Albumin (g/dL)	3.17^{a}	2.49^{b}	3.18^{a}	3.16^{a}	3.19^{a}	3.26^{a}	0.047	0.0001
Globulin (g/dL)	3.44^{a}	2.41^{d}	2.88^{c}	3.15^{b}	3.41^{a}	3.32^{ab}	0.068	0.0001
AST (U/L)	22.10^{c}	46.42^{a}	32.03^{b}	30.75^{b}	21.81^{c}	22.00^{c}	1.506	0.0001
ALT (U/L)	45.60^{c}	75.67^{a}	50.25^{b}	49.80^{b}	43.52^{d}	43.25^{d}	1.911	0.0001
Cholesterol (mg/dL)	96.60^{b}	112.33^{a}	91.90^{c}	90.36^{c}	84.50^{d}	83.68^{d}	1.773	0.0001
Triglycerides (mg/dL)	43.67^{e}	73.61^{a}	49.02^{b}	47.47^{c}	45.79^{d}	45.38^{d}	1.755	0.0001
Urea (mg/dL)	15.34^{c}	30.26^{a}	18.19^{b}	17.75^{b}	15.56^{c}	15.15^{c}	0.901	0.0001
Creatinine (mg/dL)	0.861^{c}	1.931^{a}	0.993^{b}	0.9150^{c}	0.860^{c}	0.866^{c}	0.065	0.0001

a, b, : Values in the same row with different superscripts differ significantly (P≤0.05); AST: Aspartate amino transferase, ALT: Alanine aminotransferase

Table (5). Effects of *Nigella sativa* seeds (NSS) or garlic on the oxidative status and antioxidant parameters in rabbit's liver exposed to lead acetate

Items	Dietary treatments						SEM	p-value
	Control	Lead acetate	lead+ N. sativa	lead+ garlic	N. Sativa	Garlic		
MDA (nmol/mg protein)	16.96^{d}	39.30^{a}	19.52^{b}	18.48^{c}	4.01^{e}	4.13^{e}	2.034	0.0001
GSH (µmol/mg protein)	56.78^{b}	33.36^{e}	45.60^{d}	48.06^{c}	69.00^{a}	70.70^{a}	2.242	0.0001
GST (U/mg protein)	38.68^{a}	28.11^{c}	34.95^{b}	35.25^{b}	38.40^{a}	39.00^{a}	0.636	0.0001
GPx (U/mg protein)	15.48^{a}	6.45^{c}	10.77^{b}	11.00^{b}	15.81^{a}	15.88^{a}	0.595	0.0001

a, b, : Values in the same row with different superscripts differ significantly (P≤ 0.05), MDA, malondialdehyde; GSH, reduced glutathione; GST, glutathione S-transferase; GPx, glutathione peroxidase.

Table (6). Effect of *Nigella sativa* seeds (NSS) or garlic on some carcass characters and deposition of lead (Pb, ppm) in kidney, liver and muscles in lead toxicity induced rabbits

Items	Dietary treatments						SEM	p-value
	Control	Lead acetate	lead+ N. sativa	lead+ garlic	N. Sativa	Garlic		
Carcass characters								
Dressing (%)	58.2^{a}	55.65^{b}	57.71^{a}	57.98^{a}	59.0^{a}	59.1^{a}	1.121	0.0001
Liver (%)	3.05^{b}	3.81^{a}	3.22^{b}	3.15^{b}	3.10^{b}	3.17^{b}	0.856	0.003
Kidney (%)	0.72^{c}	1.25^{a}	0.98^{b}	1.02^{b}	0.71^{c}	0.74^{c}	0.045	0.001
Heart (%)	0.354^{c}	0.586^{a}	0.415^{b}	0.404^{b}	0.350^{c}	0.352^{c}	0.035	0.002
Lungs (%)	0.658	0.665	0.662	0.658	0.66	0.661	0.124	0.425
Testis (%)	0.215	0.241	0.22	0.225	0.218	0.215	0.025	0.225
Residue of lead (ppm):								
Kidney	0.079^{d}	3.945^{a}	0.872^{b}	0.751^{c}	0.062^{d}	0.042^{d}	0.232	0.0001
Liver	0.076^{d}	3.273^{a}	0.686^{b}	0.413^{c}	0.040^{d}	0.029^{d}	0.195	0.0001
Muscles	0.036^{d}	2.946^{a}	0.618^{b}	0.400^{c}	0.014^{d}	0.019^{d}	0.175	0.0001

$^{a, b,}$: Values in the same row with different superscripts differ significantly (P≤ 0.05).

Also investigated to establish the accumulation of lead (Pb) in the kidney was the effect of *Nigella sativa* or garlic supplement in lead (Pb) toxicity induced rabbits (Table 6). A significant increase in accumulation of lead (Pb) levels in the kidney in lead acetate group at 0.05 percent (G2) may result from impaired kidney functions as a result of lead toxicity in this sample. The present study observed a reduced level of residual deposition of lead in the kidney in lead acetate at 0.05% + 2% NSS supplement (G3) and lead acetate at 0.05% + 0.8% dry garlic supplement (G4), respectively (Table 6). Compared to the lead acetate group, this ameliorating effect was more evident (0.751 ppm) with 0.05 percent + 0.8 percent dry garlic supplement (G4) treatment. These rise, however, can imply kidney function impairment. Therefore, in the case of lead exposure, urea, uric acid and creatinine may be considered as important prognostic markers of renal dysfunction (Weaver *et al*. 2003). In exposed chickens (Franson and Custer 1982) it was confirmed that kidney residues were about three times the liver residues.

The concentration of lead (Pb) in the liver of the rabbits treated with lead acetate was significantly elevated at 0.05 percent (G2) compared to the control group's rabbits. Rabbit treatment with *Nigella sativa* or garlic feed supplement reduced residual lead (Pb) deposition. It has been tested the therapeutic effects of *Nigella sativa* or garlic combing in rabbit lead poisoning. Table 8 reported the effects of progressive accumulation of lead (Pb) on the liver. Analysis of variance of liver tissue data revealed significant differences among treatment groups. The lead (Pb) levels measured in the liver were 0.076, 3.273, 0.686, 0.413, 0.040 and 0.029 (ppm) in the control group (G1), lead acetate in the 0.05 percent group (G2), lead acetate in the 0.05 percent + 2 percent NSS replacement group (G3), lead acetate in the 0.05 percent + 0.8 percent dry garlic supplement group (G4), nigella sativa seed (G5) and garlic (G6),

respectively (Table 6). Senapati et al. (2001) reported that the concentration of lead (Pb) in urine and rat feces in garlic can increase. The same authors reported that in reducing lead, the administration of garlic and lead acetate was effective. Pourjafar *et al*. (2007) documented the most effective way to detoxify the liver. In addition, a study conducted by (Hanafy *et al*., 1994) reported a decreasing in the lead burden in chickens ' muscle and liver tissues provided simultaneously to both lead and garlic.

Lead (Pb) was substantially accumulated in the muscles of lead acetate (Pb) as compared with the control group (G1) at 0.05 percent (G2). The muscle tissues were tested for deposition of lead (Pb) in experimentally lead (Pb) induced rabbits (Table 6) due to the potential health risk of lead (Pb) toxicity to humans from consuming meat from rabbits. The lead (Pb) levels measured in the muscles were 0.036, 2.946, 0.618.0.400, 0.014 and 0.019 (ppm) in the control group (G1), lead acetate at 0.05% group (G2), lead acetate at 0.05% + 2% NSS supplement group (G3), lead acetate at 0.05% + 0.8% dry garlic supplement group (G4), *Nigella sativa* seed (G5) and garlic (G6) respectively (Table 6). In residual accumulation of lead (Pb) in muscles, the NSS or garlic supplementation caused highly significant decreases in values (Pb). Garlic feeding can be used to prevent human consumption by reducing the concentration of lead (Pb) in meat from food animals grown in a polluted environment with lead (Pb) (Hanafy *et al*., 1994). The antioxidant effects of organosulfur components (as diallyl sulfide) present in garlic against toxicants (Fatma *et al*., 2009). Additional support for our findings comes from (Khan *et al*., 1994), which recorded orally administered toxic doses of lead (Pb) in the liver, muscles, and kidneys. (Hanafy *et al*., 1994) reported toxicity of garlic antagonized lead (Pb) and investigated the potential use of garlic feeding to clean up lead content from chickens exposed to natural or experimental lead (Pb) contamination. In addition, a study conducted by (Hanafy *et al*., 1994) confirmed that both lead and garlic concurrently decreased the burden of lead (Pb) in chickens ' muscle and liver tissue. Shehata, (2011) also observed that lead residue in liver, lungs, and muscles decreased significantly by 0.08%, 0.16% DL-methionine, or 2% fresh garlic. Based on the results of this analysis, it can be concluded that in minimizing the lead toxicity in rabbits, garlic is a healthy supplement.

CONCLUSIONS

The present study shows that adding 2% *Nigella sativa* seed or 0.8% dry garlic to a diet contaminated with 0.05% lead acetate provides a safe and effective way to minimize lead toxicity in rabbit diet. The obtained data suggest that the use of NSS or garlic in lead acetate-intoxicated rabbit diets may potentially enhance hepatic oxidative damage caused by lead acetate. NSS or garlic supplementation's antioxidant function was mediated by inhibition of MDA and induction of GSH and GSH-dependent enzymes, especially Se-GPx.

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