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Research Article

Effect of *Nigella sativa* Oil on Female Rats Exposed to Lead

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Abstract

Environmental pollution is among the major issues the world is facing today. Lead (Pb) contamination even, at a small amount may cause serious damage to vital organs in the body such as liver. On the other hand, medicinal plants have been used for many centuries to cure diseases, especially the black seed (*Nigella sativa*) (NS). *Nigella sativa* oil (NSO) is a great source of antioxidants and fatty acids. The objective of this research is to assess the potential use of NSO as the possible protective agent in female rats exposed to Pb using several biochemical parameters, besides the histological changes induced by Pb in liver. In this study, 40 female rats were equally allocated into 4 groups. Rats of the first group (G1) were served as normal controls. Rats of the second group (G2) were orally ingested every two days with a single dose of Pb acetate (150mg/kg/body weight) for a period of 6 weeks. Rats of the third group were exposed to Pb acetate similar to group 2 and *Nigella sativa* oil ((700mg/kg/day). Rats of the fourth group were administered *Nigella sativa* oil. Results showed that oral administration of Pb acetate exhibited highly significant increase in the levels of liver function enzymes. Histological examinations of liver sections from rats exposed to only Pb acetate showed severe alterations. This study showed that *Nigella sativa* oil attenuated the biochemical and histological changes induced by Pb intoxication.

Keywords: Environmental Pollution; Heavy Metals; Pb; Toxicity; *Nigella Sativa*; *Nigella Sativa* Oil

Abbreviations

NSO: *Nigella Sativa* Oil; ANOVA: Analysis Of Variance; MM: Millimolar; SD: Standard Deviation; ALT: Alanine Amino Transferase; AST: Aminotransferas; ALP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase; GI: Group I; G2: Group 2.

Introduction

Pollution means the addition of contaminants into the natural environment, causing damage and causing disturbance in the ecosystem [1]. These pollutants are either exogenous to the environment or natural substances but have exceeded accepted levels [2]. Pollution encompasses a wider dimensions including, chemical pollutants [3], thermal pollution [4], soil pollution [5], and biological and non-biological industrial and domestic pollution [6]. Various form of the pollution affects the human being and it is among the worse problems the globe is facing today [7].

All heavy metals are toxic at even very small concentration, they enter into the living tissues through several ways either by inhalation, eating, drinking, soil and manual handling [8]. Some heavy metals such as iron, magnesium and zinc are very important at trace amount for normal body physiology [9]. Exposure to toxins in general and to heavy metals Lead to many health problems such as disorders of the nervous system, immune disorders, cancers, kidney and liver damage and disruption of endocrine work [10]. In particular, Pb is a soft heavy metal, elastic metal element that is

extensible, white and bluish-gray, which is mostly extracted from the earth's crust. Pb toxicity occurs when build up in the body, often months or years. Even small amounts of Pb can cause serious health problems. Children under age of 6 years are more likely to have Pb poisoning, which can severely affect mental and physical development [11]. In the case of very high levels, Pb poisoning can cause death. It is difficult to detect, even people who are healthy may have high levels of Pb in their blood. Usually signs and symptoms do not appear until the accumulation of dangerous amounts is ingested [11].

Since Pb affects the vital organs of the body such as the liver and kidney, it therefore works on the precise impact on the cells in the body, and here we investigated the effect of Pb specifically on the vital organs. Sources of exposure to Pb include: industrial processes, food and drinking water, smoking [12]. Moreover, Pb poisoning occurs from several sources such as: paint houses, faucets, toys, car batteries, exhaust cars and plumbing pipes [13].

Pb toxicity in cells is shown by oxidative stress, which is caused by the imbalance between the production of free radical's (ROS) and antioxidants that work primarily on detoxification. The most prominent of these is glutathione (GSH), which is found mainly in the cell to protect it from oxidation with free radicals such as H2O2 resulting from the effect of Pb accumulation on the cell. This accumulation increases the production of ROS and decreases GSH, thus increasing the toxicity in the cell, Bring to the appearance of structural damage in cells, proteins, nucleic acids, membranes, and fat. Thus, cellular tension occurs, which does not allow the cell to perform its main functions due to the breakdown of many of its components depending on the high toxicity [13].

Herbal medicine has been known since ancient times for their therapeutic and preventive role in curing many diseases. Hence, the seeds, leaves, roots and fruits were used for the treatment of diseases and extraction of many medicinal drugs until the present age [14]. *Nigella sativa* or black seeds (*N. sativa*) were the most important medicinal plants and most commonly used because of their immune role in the treatment of toxins [15,16] and many diseases such as cancer [17], heart disease [18,19] and neurological diseases [20,21]. It is annual herb, about 20 - 90 cm long, with a straight erect stalk, delicate deep-cut leaves, blue-to-gray flowers, horns and serrated seeds [22].

The *N. sativa* was originated in the Mediterranean region and then spread to middle east like; Saudi Arabia, Iraq and sham countries (Syria, Palestine, Lebanon), turkey, Iran, North Africa such as (Egypt, Morocco, Algeria, Tunisia), parts of South Asian such as India, China. Also known in southern Europe and America [23]. The oil of the *N. sativa* commonly known as black cumin oil or black coriander oil, used as medicine for treatment of various ailments throughout the world [24]. The oil has antioxidants, anti-inflammatory and anticancer activities [23]. The present study aimed to investigate the protective effect of *Nigella sativa* oil on lead-intoxicated female rats.

Materials and Methods

Animals

Forty female Wistar rats (120 - 150g) were supplied by Mansour Scientific Foundation for Research and Development, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were kept under the condition as described by Mohamadin., *et al* [26].

Nigella sativa oil (NSO)

High quality *Nigella sativa* oil (NSO) was obtained from Adnan Subgha Centre for Natural Herbs Jeddah, Saudi Arabia. The oil was stored in the laboratory until use.

Experimental procedure

Four groups of the rats were randomly made having 10 individuals per group (n = 10/group). The NSO was administered orally as treatment for six weeks. The normal and Pb-intoxicated groups were as follows:

1. Were untreated and served as control.
2. Were orally administered a single dose of Pb acetate (150mg/kg/body weight) day after day.
3. Were orally administered a single dose of Pb acetate (150mg/kg/body weight) day after day plus *Nigella sativa* oil (700mg/kg/day).
4. Were orally supplemented with *Nigella sativa* oil (700mg/kg/day).

Samples collection

Blood sampling

At the end of the gestation period, the rats were anesthetized

with ethyl ether. Blood samples were collected from orbital plexus veins in taps other than heparin, and then extracted for 15 min at 3,000 rpm to separate serum. Blood was then collected, frozen at -80 °C and stored until used for biochemical analysis. Serum samples were used to determine the level of liver function testing (ALT, AST, ALP, GGT, total bilirubin). All biochemical parameters were measured by automatic analyzers analyzer using their specified kits.

Histological analysis

The stored liver samples were processed in a formalin tissue processor. Treatment consists of two steps, preliminary injection and dehydration. Add tissue to the tissue immersion in 10 hours buffer the format for 48 hours, then remove the fixative in distilled water for 30 minutes. Subsequently, water degradation was performed by running the tissues by alcohol classification (70%, 90% and 100%). The tissues were initially exposed to 70% alcohol for 120 minutes, then 90% alcohol for 90 minutes, then two cycles of absolute alcohol, each exposed for one hour. Subsequent dehydration eliminated the sample in several changes to xylene. It contains 50% alcohol and 50% xylene in a mixture of submerged tissues for one hour, followed by pure xylene for an hour and a half. The samples were then stained with molten paraffin wax, and then added and withheld. Paraffin sections (4 - 5 mM) were contaminated with hematoxylin and eosin [25]. Stained sections were inspected for any pathologic changes.

Statistical analysis

one- way ANOVA was applied to analyse the data and data was expressed as mean ± SD. Significant level set at $p \leq 0.05$. The statistical analysis was performed using SPSS software for windows, version 22, Armonk, NY. Data are presented using GraphPad Prism version.

Results

Clinical signs

Female rats of control group G1 and NSO group G4 did not reveal any clinical symptoms throughout study period. G2 which given Pb acetate appeared toxicity symptoms like weakness, lethargy and slow movement. While the Pb and oil treatment G3 did not notice any changes in movement, colour, or other behavioural changes throughout the duration of the experiment, except some rats that showed lethargy and slow movement sometimes, but as general the symptoms of Pb poisoning were not observed significantly throughout the treatment.

Biochemical analysis of liver functions

The results of the activities of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl Transferase (GGT) and total bilirubin in different groups studied are presented in Figures (1 to 5) and Table 1. In the control group (GI), the mean values ± SE of the enzymes ALT, AST, ALP, GGT and total bilirubin were (134 ± 1.3, 19 ± 1.2, 65 ± 3.2, 22 ± 2.2 IU/L) and (0.58 ± 0.07 mg/dl) respectively.

While in Pb treated group (G2) the mean values ±SE of the enzymes ALT, AST, ALP, GGT and total bilirubin were significantly increased ($P < 0.05$) (400 ± 2.8 *, 65 ± 4.6 *, 193 ± 5.1*, 107 ± 4.6* IU/L) (1.49 ± 0.11* mg/dl) respectively when compared with GI. In Pb treated + NSO (G3) the mean values ± SE of the enzymes ALT, AST, ALP, GGT and total bilirubin were (241 ± 2.0 * #, 38 ± 1.7 *#, 103 ± 5.4 *#, 66 ± 2.5 #*IU/L) and (0.95 ± 0.09*** # mg/dl) respectively were slightly higher when compared with GI. And in NSO treated group (G4) the mean values ±SE of the enzymes ALT, AST, ALP, GGT and total bilirubin (138 ± 0.8, 21 ± 2.3, 68 ± 4.9, 22 ± 2.3 IU/L) and (0.62 ± 0.06 mg/dl) respectively were slightly close when compared with (G1).

On other hand, the values in Pb acetate treated group (G2), were increased for (ALT, AST, ALP, GGT and total bilirubin) (400 ± 2.8*, 65 ± 4.6*, 193 ± 5.1*, 107 ± 4.6* 1.49 ± 0.11* IU/L) and (1.49 ± 0.11* mg/dl) respectively when compared with Pb treated + NSO (G3) (241 ± 2.0 * #, 38 ± 1.7 *#, 103 ± 5.4 *#, 66 ± 2.5 #*IU/L) and (0.95

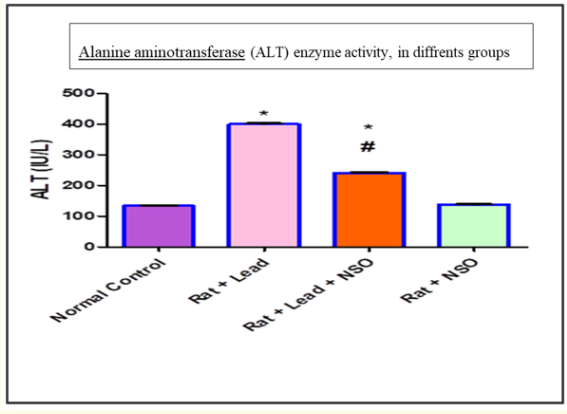

Figure 1: Mean values ± SE of Alanine aminotransferase (ALT) enzyme activity, in control group (G1), Pb treated group (G2), Pb + NSO treated group (G3), NSO treated group (G4). *P ≤ 0.001 from control, #P ≤ 0.001 from Pb.

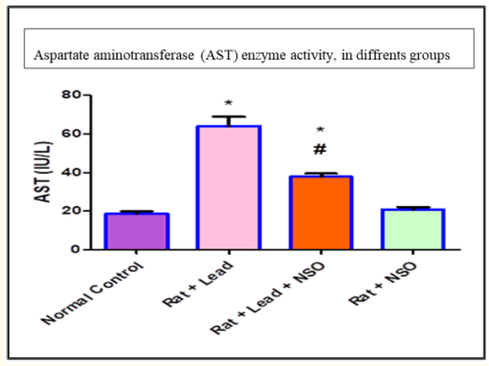

Figure 2: Mean values ± SE of Aspartate aminotransferase (AST) enzyme activity, in control group (G1), Pb treated group (G2), Pb + NSO treated group (G3), NSO treated group (G4). *P ≤ 0.001 from control, #P ≤ 0.001 from Pb.

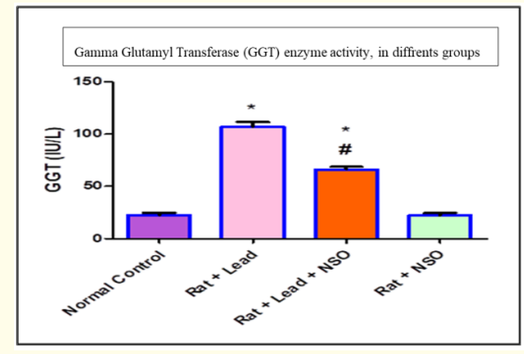

Figure 4: Mean values ± SE of Gamma Glutamyl Transferase (GGT) enzyme activity, in control group (G1), Pb treated group (G2), Pb + NSO treated group (G3), NSO treated group (G4). *P ≤ 0.001 from control, #P ≤ 0.001 from Pb.

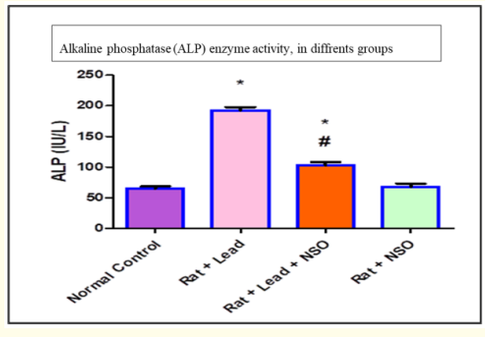

Figure 3: Mean values ± SE of Alkaline phosphatase (ALP) enzyme activity, in control group (G1), Pb treated group (G2), Pb + NSO treated group (G3), NSO treated group (G4). *P ≤ 0.001 from control, #P ≤ 0.001 from Pb.

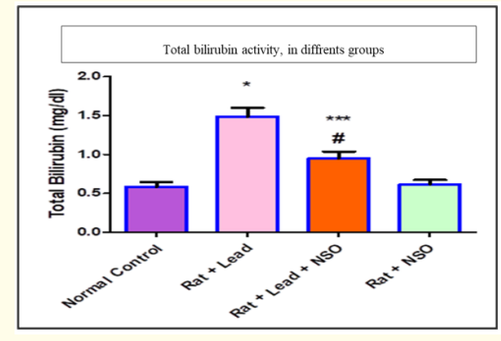

Figure 5: Mean values ± SE of total bilirubin activity, in control group (G1), Pb treated group (G2), Pb + NSO treated group (G3), NSO treated group (G4). *P ≤ 0.001 from control. ***P ≤ 0.05 from control, #P ≤ 0.001 from Pb.

± 0.09*** # mg/dl) respectively.

Histological analysis

Light microscope observations in the control group (G1)

Liver tissue in the control group (GI) revealed a normal liver composition. Figures 6A and 6B showed the liver lobules, which

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	Total Bilirubin (mg/dl)
Normal Control	134 ± 1.3	19 ± 1.2	65 ± 3.2	22 ± 2.2	0.58 ± 0.07
Rat + Pb	400 ± 2.8 *	65 ± 4.6 *	193 ± 5.1 *	107 ± 4.6 *	1.49 ± 0.11 *
Rat + Pb + NSO	241 ± 2.0 *#	38 ± 1.7 *#	103 ± 5.4 *#	66 ± 2.5 #*	0.95 ± 0.09 ***#
Rat + NSO	138 ± 0.8	21 ± 2.3	68 ± 4.9	22 ± 2.3	0.62 ± 0.06

Table 1: Effect of activated NSO on the serum levels of Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT), Gamma-glutamyl transferase (GGT) and Total Bilirubin in Pb acetate-treated in female rats.

*P ≤ 0.001 from control, **P ≤ 0.01 from control, #P ≤ 0.001 from Pb, ### P ≤ 0.05 from Pb.

are polygonal units that display plates of epithelial cells called hepatocytes radiating from central vein (CV). The liver cells were comprised of an interconnected plate such as wall brick and slab around the central vein. The central vein of each lobule was indicated by its location in the center of each lobule. Specialized cells known as Kufr (K) cells were easily identified using H and E stains and were found between endothelial sinus cells.

Light microscopy observations in Pb acetate group (G2)

The liver tissue records in the Pb group (G2) revealed that there were some changes in the liver cells such as cytoplasmic void with the larynx nuclei. In some cells, chromatin was deposited in the nuclear envelope and in some cells there was degeneration and necrosis. In addition, Koffer (K) cell numbers showed an increase (Figures 7A and 7B).

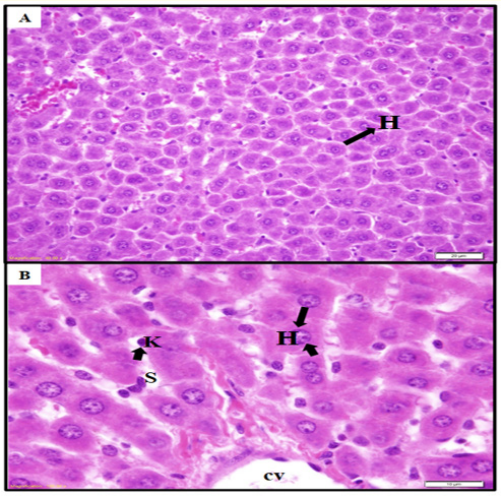

Figure 6: (A) Light microscopy micrograph of liver tissue from control group showing normal architecture, normal hepatocytes (H). (B) with central nuclei, which forms hepatic cords radiating around central vein (CV), with blood sinusoids (S) with normal Kupffer cells (K). (a) (H and E stain X 40), (b) (H and E stain X 60).

Light microscopy observations of liver in Pb and NSO group (G3)

Examination of the liver in Pb acetate and NSO (G3) group revealed that liver tissue appeared in the normal control group, where the liver cords appear normal and circulate around the central vein. do. Liver cells were usually shown with round nuclei and were mainly found in the cytoplasm, but to some extent there were some liver cells, including the cytoplasm that remained in space and weak stained nuclei, sinusoids in the blood and the Kupffer cells were shown as normal (Figure 8A and 8B).

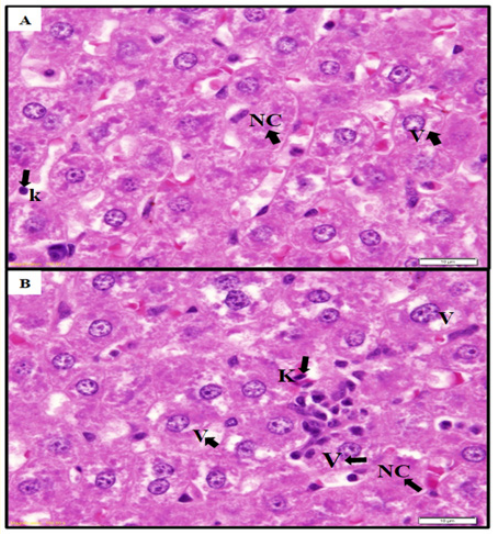

Figure 7: (A) Light microscopy micrograph of normal liver tissue (B) Micrograph from Pb treated group showing changes in the hepatocytes, which appeared in vacuolation (V) of cytoplasm and necrosis (NC) in some cells and increasing in size and numbers of Kupffer cells (K). (a) (H and E stain X 100), (b) (H and E stain X 100.

Light microscopy observations in NSO group (G4)

The liver of the NSO (G4) group revealed a normal structure similar to that in the control group (GI), where the liver cells, which appeared normal with the nuclei in the center, rounded, and that the sinusoids (S) arranged in between the hepatic cords. Kupffer cells were purified in their normal number (K) (Figs 9A and 9B).

Discussion

When Pb accumulates in the body, it is poisoned for months or years later. Even a small amount of lead can cause serious health problems. Children are more likely to get poisoned, which can severely affect mental and physical development. At very high levels, Pb poisoning can lead to death [26]. *N. sativa* is an important

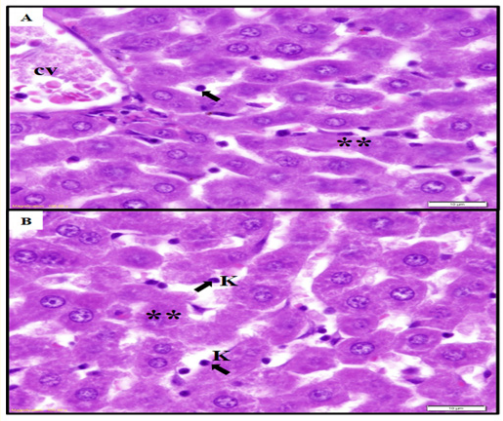

Figure 8: (A) light microscopy micrograph of liver tissue from Pb and NSO group showing some improvement of the hepatocytes, which appeared normally in the hepatic cords, around the central vein (CV). (B) Some cells still showing changes (**) in the form of necrosis and increasing in Kupffer cells (K). (a) (H and E stain X 100), (b) (H and E stain X 100).

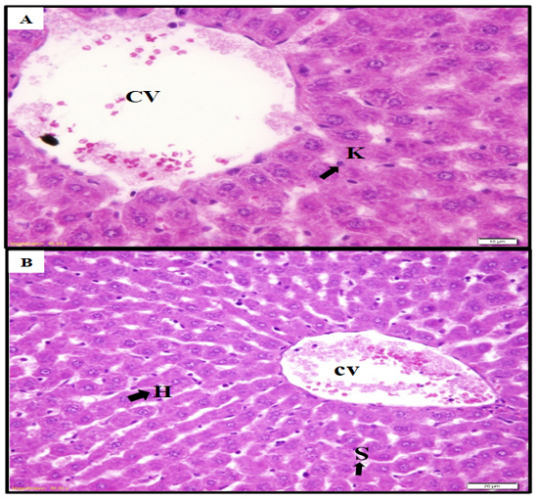

Figure 9: (A) Light microscopy micrograph of liver tissue from NSO administered group showing improvement of hepatocytes (H) with central nuclei which arranged in hepatic cords around central vein (CV). (B) Normal number of Kupffer cells (K), and normal sinusoids (S). (a) (H and E stain X 60), (b) (H and E stain X 40.

medicinal plants, commonly known as blackhead or black coriander which belong to Ranunculaceae family. *N. sativa* has greater medicinal importance in Islamic medicine, everything except death is known as cure. The results of this study showed that Pb acetate caused biochemical alterations, toxicity and histological changes in the liver. The levels of the enzymes e.g., ALT, AST, GGT, and ALP activities were increased in the present study, after administration of Pb acetate then decreased approximately to the control level when administering with NSO. The results could be due to the protective effect of NSO. Research carried out by Randhawa., *et al.* [27] showed the protective impact of thymoquinone (TQ), the main active ingredient in volatile *N. sativa* seed oil, against Pb-induced liver injury. In a similar study, Ahmed and Hassanein [29], studied the cardio-protective effect of *N. sativa* oil (NSO) on Pb induced cardio toxicity. The authors found that the Pb intake induced a significant increase ($p < 0.001$) in malondialdehyde (MDA) in treated group as compared to control. It has been reported that the protective role of *N. sativa* in several natural and chemical toxins, including Pb, is in line with current research that causes AST, urea, creatinine, total cholesterol and significant increase [10,29].

Morphological modifications in the liver that follow acute Pb exposure rely on the amount and moment at which these modifications are observed and may differ from dilation of the rough endoplasmic reticulum with loss of ribosomes to hepatocellular necrosis [17]. In addition, liver is the largest gland in the human body and performs many functions including glycogen storage, blood glucose release, and biotransformation process. The results of the histopathological examination showed that administration of Pb, to female rats resulted in a clear effect in hepatocytes (H) with the appearance of a vacuolation (V) in the cytoplasm, an increase in the size and number of Kupffer (K) cells, and necrosis (NC) in many cells. The present results showed that administration of *N. sativa* oil to Pb-intoxicated rats significantly repaired the damaged areas which were affected due to Pb toxicity. Similarly, Radwan and Mohamed [30] explored the modulatory effect of *N. sativa* on the therapeutic potential of mesenchymal stem cells (MSCs) against irradiation-induced liver damage in rats. The authors observed that combined NSO/MSCs therapy provided more beneficial tissue repair comparable to MSCs alone [30].

Conclusion

The present study concerned with toxicity of the Pb, which cause severe damage to the liver structure and functions. The treatment of the liver injured by Pb toxicity was significantly decreased by the administration of *N. Sativa* oil. The enzymatic function in the liver was also restored to the normalancy and showed the recovery of the damaged liver. The treatment significantly repaired the damaged areas which were affected due to Pb toxicity. In short, the *N. Sativa* oil showed a great medicinal effect to recover the damage liver and could be used as treatment against the Pb toxicity.

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