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L-CARNITINE NANOPARTICLES EFFECTS ON PHYSIOLOGY AND HISTOPATHOLOGY CHANGES INDUCED BY LEAD ACETATE IN MALE RATS

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ABSTRACT

The study's objective is to evaluate the enhanced effect of the L-Carnitine and Nano L-Carnitine on body weight, oxidative stress biomarkers (SOD and NO), lipid profile, and the histopathologic features of the brain (cerebrum) in male rats after inducing toxicity by lead acetate.

Material and methods six groups of adult Wistar albino rats (10 per group) were intubated daily as follows: The first group (control group): received orally Distilled water. The second group: received orally lead acetate at a dose of 30 mg/kg of body weight daily for 30 days. Third group: received a combination of (lead acetate 30mg/kg B.W. for 30 days + L-carnitine 100mg/ kg B.W. daily for two months). Fourth group: received a combination of (lead acetate 30mg/kg B.W. for 30 days +Nano L-carnitine 100mg/ kg B.W. daily for two months). The fifth group: received L-Carnitine orally at a dose of 100mg/ kg B.W. Daily for two months. The sixth group: Animals received L-Carnitine -N.P.s orally at 100mg/ kg B.W. daily for two months.

The results showed that exposure to Lead Acetate caused a significant decrease in body weight in males' rate was observed in group lead acetate. At the same time, the lipids profile study showed a significant increase in triglyceride, total cholesterol, low-density lipoprotein (LDL), and a significant decrease in high-density lipoprotein (HDL) after exposure to Lead Acetate. While at a dosage of 100mg/kg, L-Carnitine significantly increased HDL-C levels and decreased T.G., T.C., VLDL, and LDL lipid levels in males. This lipid-lowering effect might be due to its antioxidant potential. The lead treatment increased the oxidative stress and reactive oxygen species (ROS), which would be cleared by a decrease in superoxide dismutase (SOD), and nitric oxide (NO). The histopathological study showed significant changes in the brain (cerebrum) and Showed Alzheimer's type II astrocytes, characterized by double nuclei surrounded by clear space. Showed intracellular and extracellular vacuoles showed degeneration and necrosis of cortical neurons. At the same time, some of these changes were improved to approximately normal levels after treatment with L-Carnitine (L.C.). The most changes regained the normal levels after treatment with L-Carnitine nanoparticles (LC-NPs) in the experiment.

Keywords: nanoparticles, chitosan, L-carnitine, lead acetate, lipids profile, brain, oxidative stress.

1. INTRODUCTION

Biotechnologically generated medications, such as peptides and proteins, have recently focused on drug research efforts (1). The traditional pharmacokinetic parameters, including absorption, distribution, metabolism, and elimination, are frequently used to track the fate of biotechnologically produced pharmaceuticals following in vivo delivery. (2). Such barriers necessitated the development of novel delivery technologies capable of increasing the oral bioavailability of therapeutic proteins from less than 1% to at least 30-50% (3). Many benefits exist for nanoparticle delivery systems in vivo, including high stability, long-term payload capacity release, and the ability to permeate via tiny capillaries and cellular compartments (4).

Chitosan is suggested as good functional material in numerous sectors because of its outstanding biocompatibility, biodegradability, non-toxicity, adsorption capabilities, and so on (5). Chitosan has received a lot of interest as a possible polysaccharide resource with various industrial and medicinal uses (6). It is a preferred carrier in drug delivery systems and is now employed in oral drug delivery (7).

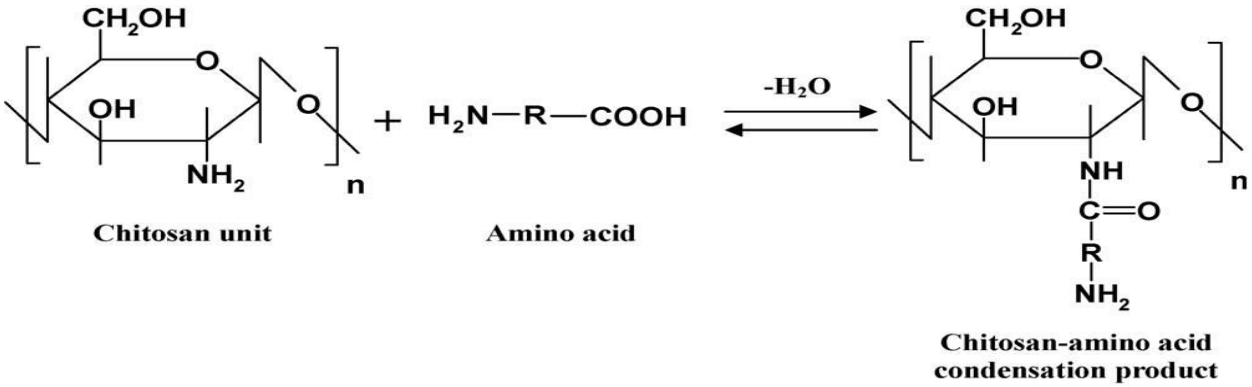
Carnitine (β-hydroxy-γ-trimethylammonium butyrate) is a hydrophilic quaternary amine required for energy metabolism. Carnitine's primary role is to transport long-chain fatty acids to mitochondria for subsequent beta-oxidation; Carnitine also binds acyl residues derived from amino acid intermediate metabolism and aids in their removal as a scavenger. (8). Many researchers have indicated that L.C. has a crucial function in many organs' oxidative and antioxidative balance. (9,10). L-carnitine prevents oxidative damage and is vital for maintaining cellular energy balance (11). it is also essential in regulating cerebral functions and the aberrant regulation of genes involved in carnitine production and mitochondrial carnitine transport. (12).

Because lead is a non-biodegradable heavy metal, its concentration in the atmosphere rises with increasing danger as a result of its long-term usage (13). Industrial operations such as lead smelting, pottery and boat building, lead-based painting, lead-containing pipes, battery recycling grids, the armaments industry and pigments are the most prevalent sources of lead exposure in humans. (14). As a toxic metal, lead has a detrimental effect on almost everybody's organs (13). When lead poisoning affects the neurological system, it is the most affected target. (15, 16). For every system that lead acetate comes into contact with (hematopoietic, renal, reproductive & central nervous), it causes oxidative stress that damages every part of the body. (17). The testes and the nervous system are two of the most harmful interactions with the body. (15,18)

2. MATERIALS AND METHODS

Synthesis and Characterizations of LC-NPs
Chitosan-carnitine acid adduct (Cs-Ca) synthesis:

According to references, these steps were used to make chitosan-l-carnitine adduct (19,20,21). 1% of l-carnitine and Chitosan were combined in equal amounts using Dean-Stark (Clevenger) apparatus. The condensation process was carried out in the presence of xylene until the water had been separated. An electric oven was used to dry and weigh the Chitosan amide product after separating and washing it with three different solvents (methanol, hot distilled water, and ethanol).



Nanoparticles made of Chitosan and carnitine were synthesized

Synthesis of Cs-Ca NPs, by ionic gelation method, was used using TPP and Cs-Ca adduct. For six hours, Cs-Ca (1 mg/ml) and TPP solution were mixed at ambient temperature with constant stirring at a ratio of 1: 2.5 (w/w %) in an acetic acid solution (1 percent w/v). TPP triggered ionic gelation of Cs-Ca/TPP nanoparticles. This was the first step in the process. Separation, washing, and drying of these nanoparticles resulted in the precipitate being re-suspended in water and drying.

Characterizations of L-carnitine-chitosan nanoparticles
These characterization tests were done in the material research laboratories at the ministry of sciences and technology environment and water research and technology director (EWRTD).

XRD Measurement
The X-ray diffraction (XRD) analysis was performed using an XRD system (Phillips PW 1830) operated at a voltage of 40 kV and current of 20 mA to determine the crystallinity, metallic nature, and cubic structure of the tested samples. Analysis was carried out in the department of Materials Research, Ministry of Science and Technology, Iraq. (22)

HPLC ((high-performance liquid chromatography)
Chitosan, Nano L-Carnitine, and other natural chemicals may be separated and analyzed using HPLC, a powerful technology that is frequently utilized. There are several benefits to using this method because of its simplicity and sensitivity (23). Nano L-Carnitine may also be separated and quantified using this method. Mixtures have been separated using a broad variety of fixed and mobile phase configurations. C18 column, solvent supply, sample injection, detector and recorder are included in the conventional HPLC equipment. The stationary phase's retention, peak form, and selectivity are all taken into account while choosing the best column. In addition to protecting analytical columns from sample contaminants, guard columns enhance the life of the column itself (24).

Animal of the study

The present study was conducted in the college of Veterinary medicine –at Basrah University, in the animal house of the department of physiology. A total number of 60 male adult albino rats (*RattusRattus*), with an average weight between (212±10g) and the ages (8- 10) weeks. They were housed for two weeks for an adaptation before the experiment. Every ten animals were housed in an individual plastic cage measured as 15x35x50cm. They were fed *ad libitum* with the meal of standard pellet of diet supplied and free access to water to drink, and they were kept under the condition of temperature (22-25) C° and light, the regime of 14hours of light, and 10 hours, of darkness.

3. THE EXPERIMENTS DESIGN

Animals in the study were divided into six groups. Each group consists of 10 male rats used for the design experiments as the following:

1- Group-1(control Group): received orally Distilled water
2- Group -2 (lead acetate Group): received orally lead acetate at a dose of 30 mg/kg of B.W daily for 30 days. Depended on LD50.
3- Group 3: (lead acetate 30mg/kg B.W..for 30 days + L-carnitine (100mg/ kg B.W. daily for 2 mouths)
4- Group 4: lead acetate 30mg/kg B.W. for 30 days +Nano L-carnitine (100mg/ kg B.W.. daily for 2 mouths)
5- Group 5 (L-carnitine): Animals received L.C. orally at a dose of 100mg/ kg B.W. daily for two mouths
6- Group 6 (Nano L-carnitine): Animals received LC-NPs orally at a dose of 100mg/ kg B.W. daily for two months.

Note: - all experiments proceed for two months.

Body weight measurements

At the beginning of the experiment, the initial body weight of adult rats was recorded and then obtained until the end of the experiment; Final body weight(gm) was also recorded to calculate Mean body weight gain.

Sample collection

At the end of the experiment, animals were sacrificed by anesthetized, placing them in a closed jar containing cotton sucked with diethyl ether anesthesia. Then blood sample was collected via cardiac puncture according to the Hoff method(25). by using a 5ml disposable syringe, (5)ml of the blood collection were put in a non-heparinized plane tube to be centrifuged at (3000 rpm for 15 minutes) to obtain the serum, which is then transferred to epndroffe tubes and stored at (-4C) for biochemical parameters examination (Serum total cholesterol (T.C.), triglyceride, high-density lipoprotein (HDL-C), Low-density lipoprotein LDL-C according to (26). And The oxidant and antioxidant parameters: Superoxide Dismutase (SOD) Activity and Nitric Oxide (NO)**.** Also, the reproductive organs (brain) were collected for histological examination.

Statistical analysis

For data analysis, used the statistical software package Statistical Package for Social Scientists (SPSS version 18.0). A one-way ANOVA with an LSD post hoc test revealed a significant difference in group mean values. Whenever possible, paired t-tests were used to compare standards. When the p-value was less than $p<0.05$., the results were considered significant. (27)

Results

Carnitine-loaded Chitosan nanoparticles (LC-NPs)

In the ionic gelation method, the nanoparticles were formed through interactions between the positively charged Chitosan and negatively charged phosphate groups of TPP.

Characterization of L-Carnitine nanoparticles (LC-NPs)

Different spectrophotometric techniques like XRD, and HPLC, analysis were used to investigate the elemental composition, crystalline nature, functional group, and stability of synthesized LC-NPs.

X-Ray Diffraction analysis (XRD)

The XRD measurement for the prepared Chitosan nanoparticles (CNPs) and L-Carnitine nanoparticles (LC-NP) to confirm the results obtained by SEM and to determine the size of the nanoparticles as shown in figures (1). There were six distinct diffraction peaks at 2θ values = 19, 32, 35, 38, 44, and 57 for LC-NPs, as shown by the results of the experiment.

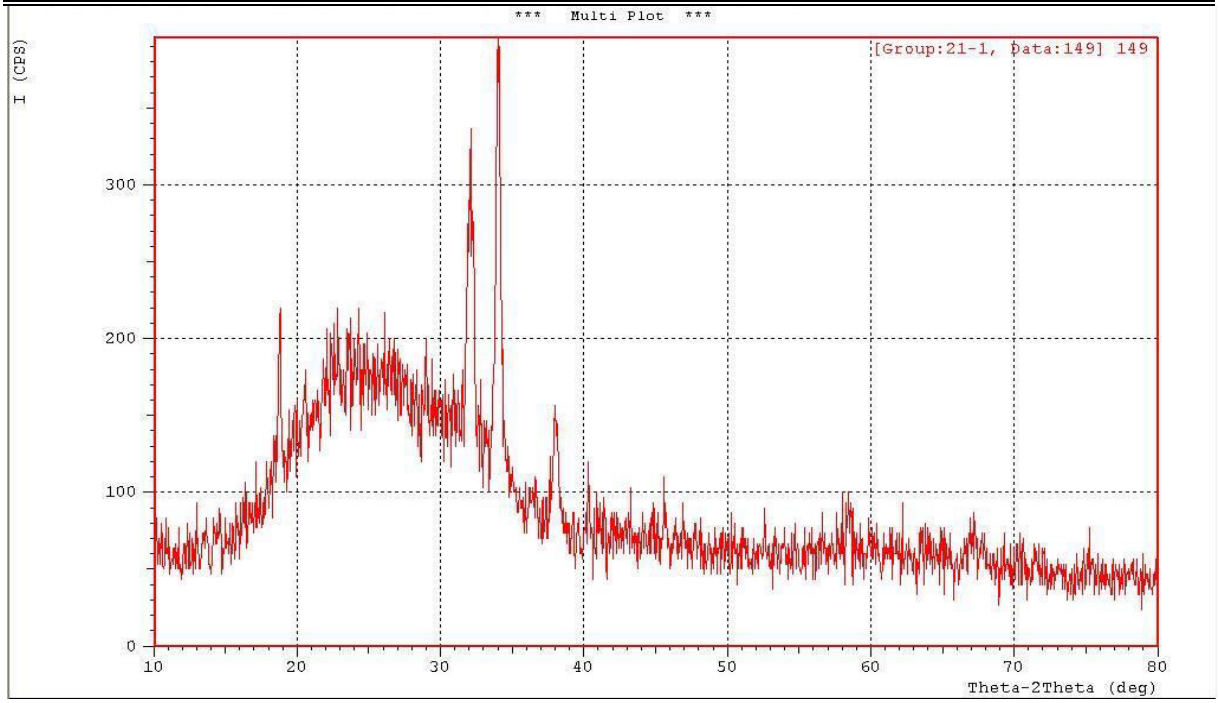


Figure (1): XRD of biosynthesized Chitosan Nanoparticles

4. HPLC ((HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)

A liquid-phase separation technique, high-performance liquid chromatography (HPLC) is a method of separation. High-pressure, high-speed injection of liquid samples into a solvent stream carries them to a high-resolution packed column, where they are separated into components by the solvent-column chemistry interaction. A Photo-Diode Array (PDA) examines the absorption spectra of each chemical ingredient at a high rate of acquisition, allowing for the detection and quantification of components (Mass Spectrometry can also be used). It was also used to determine the enantiomeric purity of L-carnitine and nano L-carnitine.

HPLC was performed to evaluate the quality and quantity of LC-NPs chitosan. A blue peak with a retention time of 8.07 min represents Chitosan. Total run time was 10 min in(fig.2(A)).

Large peak with Retention time was 8.14 min representing Chitosan, with the peak at 5.12 min representing L-carnitine; total run time was 15 min. in(fig.2(B)).

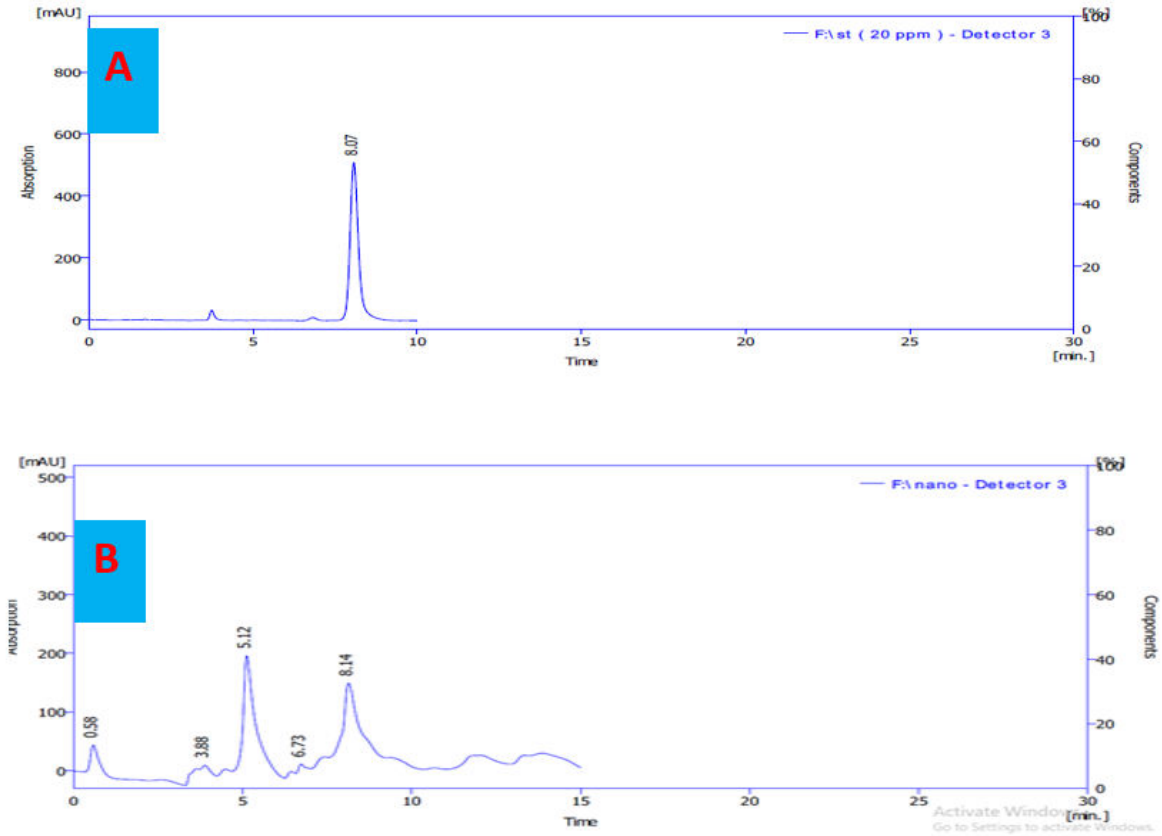
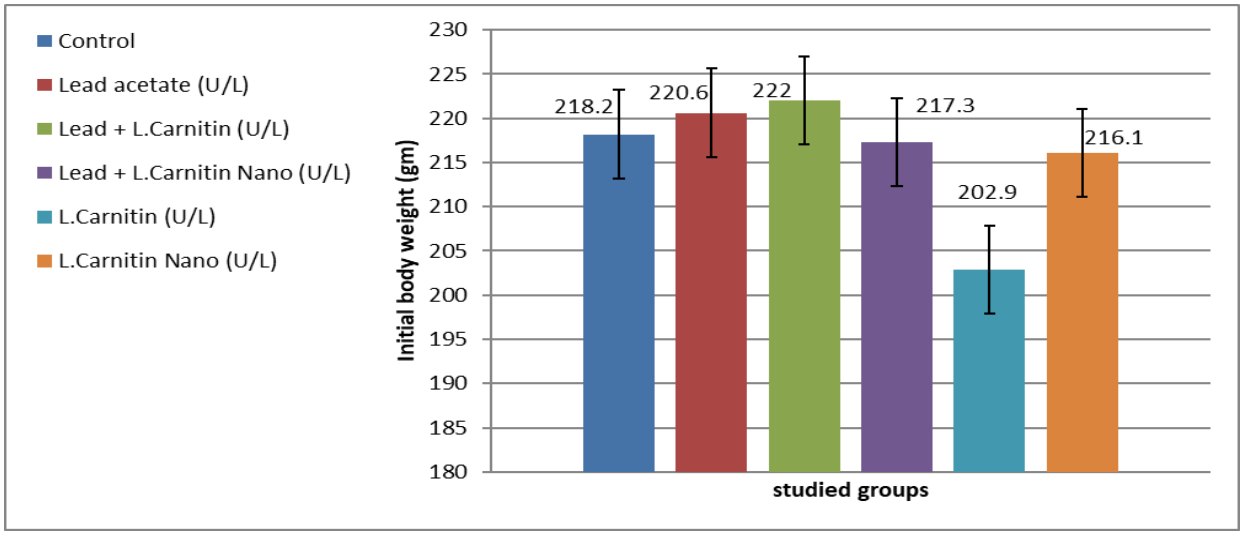


Figure (2) Chromatograms of (A) Chitosan (B) Nanoparticles L- carnitine

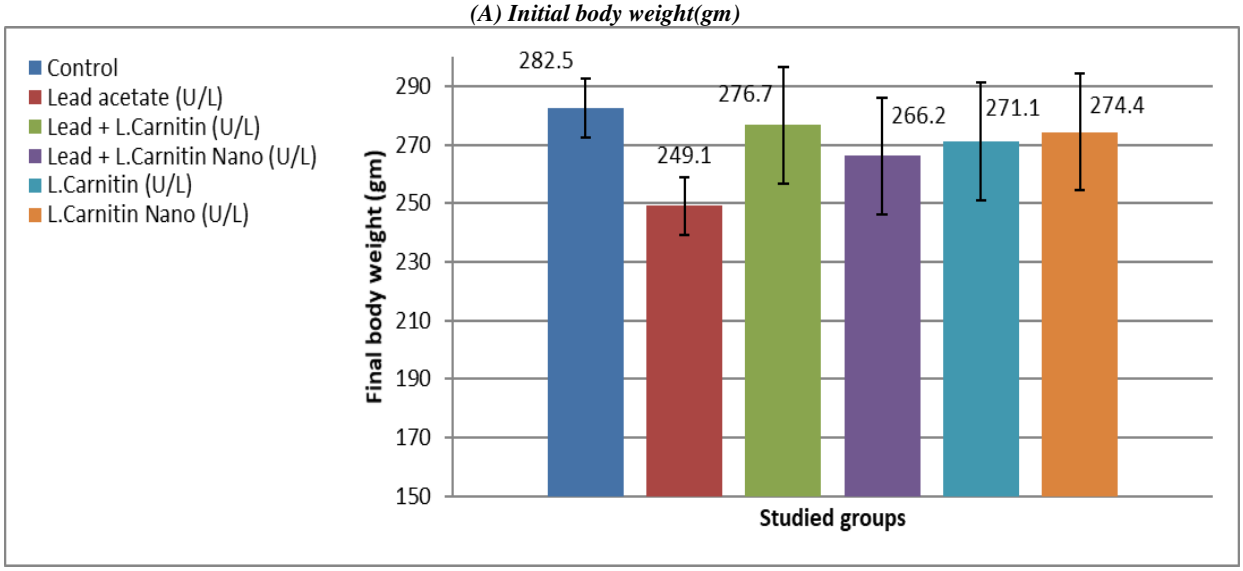
Effect of Lead acetate and its combination with L-Carnitine and Nano L-Carnitine on initial, final, and body weight gain in adult male rats.

Figure (3. A) illustrates the effect of lead acetate, L-carnitine, Nano L-carnitine, or their combination with lead on initial and final body weight in adult male rats. There was a significant decrease (P<0.05) in final body weight (3.B) was observed in treated groups of lead acetate, lead acetate+ l-carnitine, and lead acetate + Nano L carnitine with mean values were (249.1gm), (276.7gm), and (266.2gm) respectively as compared with Control, L carnitine, and Nano L-Carnitine group (282.5gm), (271.1gm) and (274.4gm) respectively.

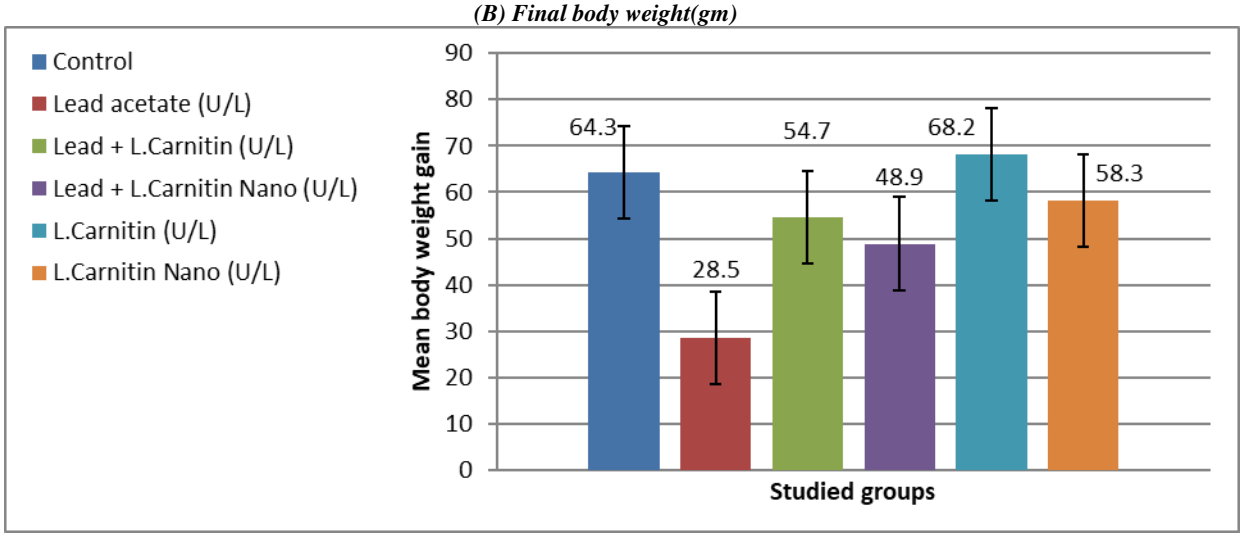
A significant decrease (P<0.05) in body weight gain was observed in the lead acetate group (28.5gm) as compared to control (64.3gm), and L Carnitine (68.2gm), and Nano L carnitine (58.3gm) groups as shown in figure(3.C). Besides a non-significant (P<0.05) increase in final body weight, weight gain was observed in group lead acetate + L carnitine and lead acetate + Nano l carnitine compared with group control in Figure 3. B and C.



(A) Initial body weight(gm)



(B) Final body weight(gm)



(C) Mean body weight gain (gm)

Figure (3) shows the effect of lead acetate, L-Carnitine, and Nano L-Carnitine or both on (A) initial body weight, (B) final body, and (C) body weight gain in adult male rats.

5. BIOCHEMICAL PARAMETERS

Lipid profile

Table (1) showed a significant($P<0.05$) increase in serum T.C., T.G., LDL-C, and VLDL-C concentration, and a decrease in serum HDL-C was recorded in lead acetate compared with the control group. However, a significant($P<0.05$) decrease in serum T.C., T.G., LDL-C, and VLDL-C concentration and an increase in serum HDL-c was recorded in lead acetate+ L-carnitine and lead acetate +Nano L carnitine groups compared with lead acetate group. While there was no significant difference in serum T.C., T.G., LDL-C concentration was recorded in the lead acetate+ L-carnitine and lead acetate +Nano L carnitine groups compared with the control group.

However, no significant difference in serum HDL-C was recorded in the lead acetate +Nano L carnitine group compared with the control group.

Finally, no significant difference in serum T.C., T.G., LDL-C, VLDL-C, and HDL-C was recorded in L-carnitine and Nano L-Carnitine groups compared with the control group.

Table (1): shows the effect of lead acetate, L-Carnitine, and Nano L-Carnitine or both on the serum lipid profile of male rats.
(Mean ± SD) n=10.

Groups	parameters (Mean ± S.D.)				
	T.G. (mg/dl)	**T. Cholesterol** (mg/dl)	**HDL** (mg/dl)	**VLDL** (mg/dl)	**LDL** (mg/dl)
Control	52.3 ± 2.61^{dc}	68.2 ± 1.97^{b}	37.0 ± 3.51^{ba}	10.4 ± 0.52^{dc}	20.7 ± 4.4^{cb}
Lead acetate (U/L)	70.8 ± 2.56^{a}	90.3 ± 2.0^{C}	28.0 ± 3.04^{d}	14.1 ± 0.51^{a}	48.0 ± 2.56^{a}
Lead + L.Carnitin (U/L)	62.4 ± 3.20^{b}	71.1 ± 11.8^{b}	30.9 ± 2.59^{dc}	12.4 ± 0.64^{b}	27.7± 12.26^{b}
Lead + L.Carnitin Nano (U/L)	55.8 ± 2.87^{c}	73.0 ± 1.75^{b}	33.0 ± 3.60^{cb}	11.1 ± 0.57^{c}	28.8 ± 3.80^{b}
L.Carnitin (U/L)	51.1 ± 2.58^{d}	66.8 ± 2.75^{b}	38.3 ± 3.24^{a}	10.2 ± 0.51^{d}	18.2 ± 4.44^{c}
L.Carnitin Nano (U/L)	50.7 ± 2.41^{d}	66.6 ± 2.84^{b}	39.9 ± 3.52^{a}	10.2 ± 0.48^{d}	16.5 ± 5.47^{c}
LSD	**2.44**	**4.73**	**2.93**	**0.48**	**5.68**

*The different small letters de not show significant differences at (P<0.05)

SOD and Nitric oxide

The results in Table (2) revealed a significant (P<0.05) decrease in serum SOD. Levels were recorded in the lead acetate group compared with the control group.

However, a significant (P<0.05) increase in serum SOD. Levels were recorded in Lead acetate+ carnitine and Lead acetate +Nano L carnitine compared with the lead acetate group. No significant difference in serum SOD levels was recorded in Lead acetate +Nano L carnitine compared with the control group, and there was no significant difference found between them. A significant (P<0.05) decrease in serum Nitric Oxide levels was recorded in the Lead acetate treated group compared with the control group, while no significant difference was observed in Nitric Oxide levels in the Lead acetate +Nano L carnitine and L Carnitine only group compared with the control group.

Table (2) Effect of Lead acetate, Lead + L.Carnitin, Lead + L.Carnitin Nano L.Carnitin Nano and L.Carnitin on SOD and Nitric oxide.
(Mean ± SD) n=10.

Groups	parameters (Mean ± S.D.)	
	NO (µmol/mg)	**SOD** (U/mg)
Control	4.5 ± 0.54^{ab}	13.7 ± 1.73^{abc}
Lead acetate (U/L)	2.5 ± 0.43^{c}	10.7 ± 1.32^{d}
Lead + L.Carnitin (U/L)	3.07 ± 0.46^{c}	12.1 ± 1.53^{dc}
Lead + L.Carnitin Nano (U/L)	4.2 ± 0.26^{b}	12.6 ± 1.21^{bc}
L.Carnitin (U/L)	4.6 ± 0.61^{ab}	14.0 ± 0.79^{ab}
L.Carnitin Nano (U/L)	5.01 ± 1.01^{a}	14.4 ± 1.06^{a}
LSD	**0.43**	**1.17**

*The different small letters de not show significant differences at (P<0.05).

Histopathological Analysis

The Cerebrum cortex

The cerebral cortex of the control rat brain was examined microscopically and revealed the normal structure and layered appearance of the cerebral cortex designated 1-6 as follows: 1 – the outside molecular layer; 2 – the external granular layer; 3 – the exterior pyramidal cell layer; 4 – the interior granular layer; 5 – the ganglionic layer (Internal pyramidal cell layer) Layer 6 is a multiform layer. Fig (4)

The Microscopic examination of sections in cerebrum cortex of Lead Acetate treated rats. The treated rats were administrated 30mg/kg body weight of Lead Acetate for 30 days. Showed Alzheimer's type II astrocytes, characterized by double nuclei surrounded by clear space. Showed intracellular and extracellular vacuoles showed degeneration and necrosis of cortical neurons. Disruption of the typical arrangement of cell layers was seen. Fig 5 and Fig 6.

The Microscope examination of the Section in the rat's cerebrum, which administrated lead acetate for 30 days with L-carnitine for 60 days, shows L-carnitine led to the recovery of the congested area with necrosis and normal fibre appearance. Fig 7.

The Microscopic examination of a section in a rat's cerebrum which administrated lead acetate for 30 days with L-carnitine for 60 days shows the cells and normal arrangement of cerebrum layers typically. Fig 8.

Histopathological microscopic features of H&E-stained slides of tissue cerebrum cortex of rat administrated L-Carnitine, and Nano L-Carnitine showed standard architecture, and distinct layers of the cerebrum after treatment with L-carnitine(100mg/kg), for 60 days. Fig 9 and Fig 10.

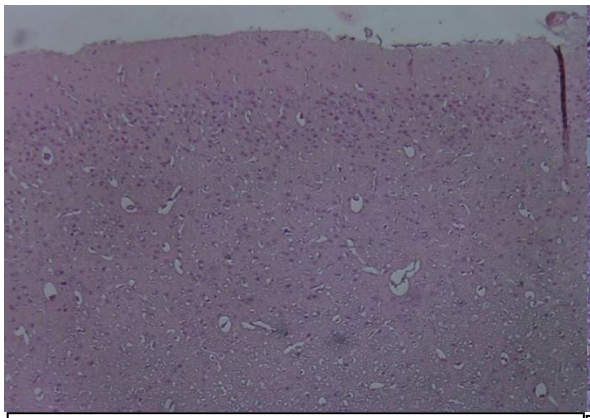


Figure (4) The cerebrum cortex in the control group showed no histopathological changes (H&E stain 100X).

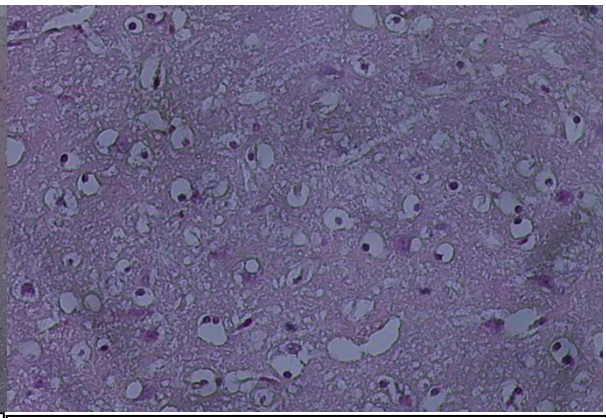


Figure (5) Photomicrograph of sections in cerebrum cortex of Lead Acetate treated rats. The treated rats were administrated 30mg/kg body weight of Lead Acetate for 30 days. (H&E stain 100x).

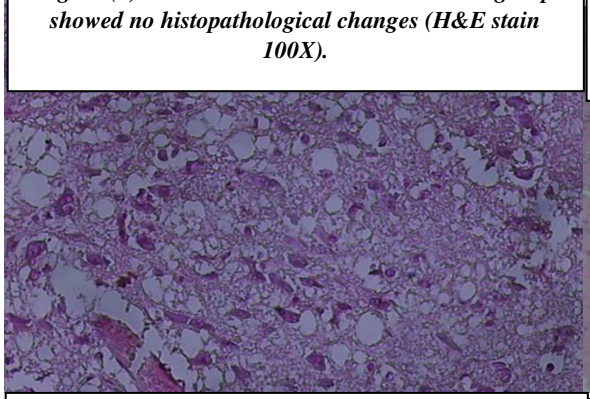


Figure 6. Photomicrograph of sections in cerebrum cortex of Lead Acetate treated rats. The treated rats were administrated 30mg/kg body weight of Lead Acetate for 30 days. (H&E stain 100x).

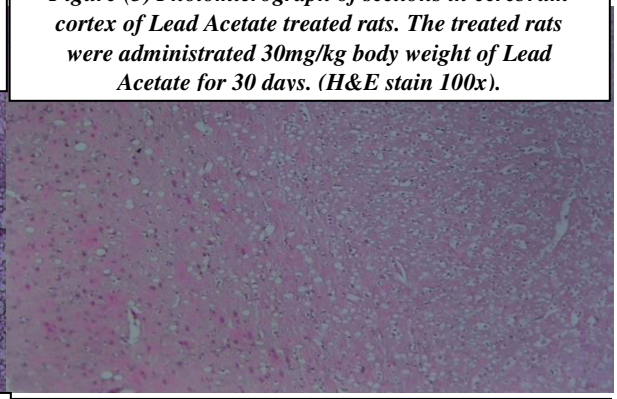


Figure (7) Photomicrograph section of rat's cerebrum administrated lead acetate for 30 days with L-carnitine for 60 days. (H&E stain 100x).

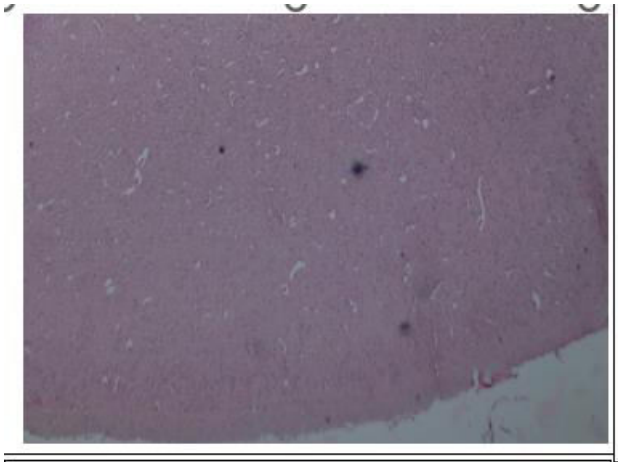


Figure (8) Photomicrograph section of rat's cerebrum administrated lead acetate for 30 days with Nano L-carnitine for 60 days. (H&E stain 100x).

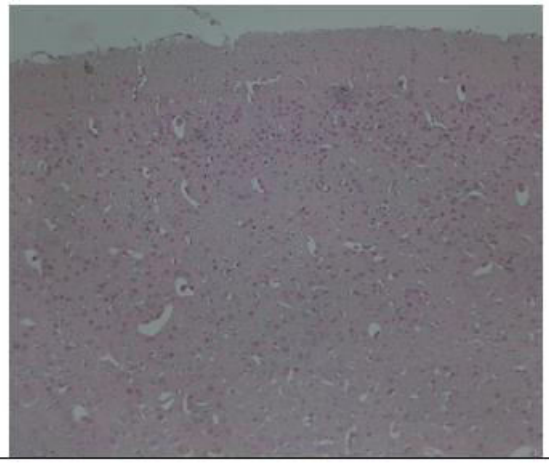


Figure (9) Histopathological microscopic features of H&E-stained slides of tissue cerebrum cortex of rat administrated L-Carnitine (100mg/kg) for 60 days. (H&E stain 100x).

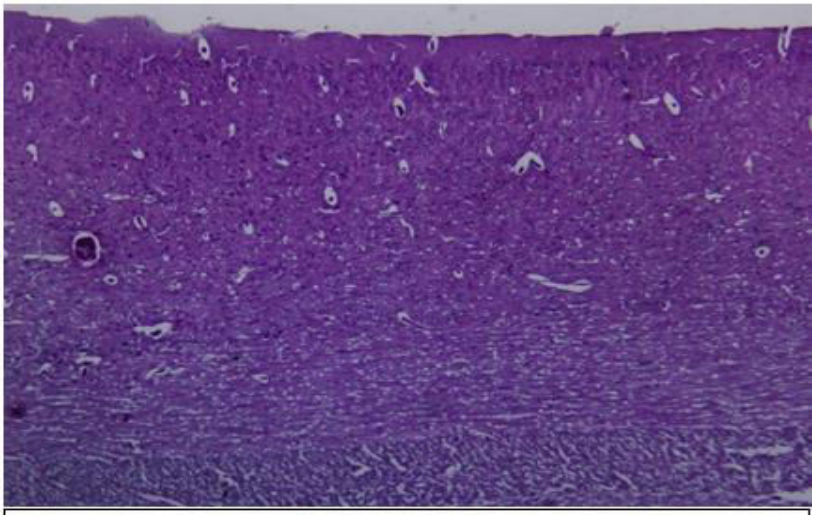


Figure (10) Photomicrograph of sections in cerebrum cortex of rat administrated Nano L-Carnitine. Shows typical architecture, and typical layers, after treatment with Nano l-carnitine(100mg/kg), for 60 days (H&E stain 100 X).

6. DISCUSSION

Characterization of L-Carnitine-chitosan Nanoparticles

XRD patterns of LC-NPs from an aqueous solution are displayed in this image (Fig. 1). The Chitosan face-centered cubic index (fcc) is used to identify the most prominent peaks. X-ray powder diffraction may offer information on unit cell dimensions when used for crystal phase identification. It is a rapid analytical method. XRD analysis for LC-NPs showed six distinct diffraction peaks at 19, 32, 35, 38,44, and 57. These results are compatible with (28). A structural investigation of chitosan magnetic nanocomposite (CMNC) and functionalized CMNC is performed using XRD. The findings demonstrate that the nanoparticles have six distinct peaks, indicating that they are nanoparticles (29). Magnetite chitosan produced in several ways has yielded similar results (30). Chitosan N.P.s are visible in the XRD diffractogram (2θ = 19.5 and 21.0) because

their crystalline peak intensity reveals their solid-state characteristics (31). Specifically, the 20 values = 30.12, 35.43, 43.22, 57.19, and 62.80 diffraction peaks of the crystallized cubic magnetite structure. Narrower peaks show the crystal structure. This sample's XRD pattern was classified as the bulk magnetite phase (32). The diffraction pattern of CS NPs reveals a prominent peak at 20 with a range of 15-24, which agrees with the results of (33). The XRD pattern of CS NPs revealed broad diffraction peaks at 20 values of 19.85°, which are distinctive fingerprints of CS NPs. This is crucial to note. (34). Most liquid-phase synthesis techniques could regulate the shape and size of N.P.s and nanostructures pretty effectively (depending on the method used). Chromatography and high-performance liquid chromatography (HPLC) are chemical purification and size separation procedures. The Retention time of 8.07 min represents chitosan. This result agrees with (35). Chitosan dimer's retention period is delayed when the acetyl group is removed, resulting in an increased affinity for the column. The HPLC spectrograms' Retention time was 8.14 min representing chitosan, with the peak at 5.12 min representing L-carnitine. This result agrees with (36).

The effect of lead acetate, L-Carnitine, and L-Carnitine loaded chitosan-nanoparticles on body weight gain and lipids profile and oxidize stress(SOD and NO)

The present study results showed in figure (3) that administration of Lead Acetate showed a significant decrease in body weight gain in rats compared to control, L Carnitine, and Nano L carnitine groups. We notice a significant decrease in rat weight in this study, resulting from reduced food intake and an increased catabolic state. Decrease of food intake and increased catabolic state due to the high duration of exposure to lead acetate and lack of balance in the metabolism resulting from changes in the zinc-dependent enzyme (37). Simultaneously, the elevation of serum and tissue ROS levels is associated with a decreased SOD concentration and a decline in NO. Various mitochondrial antioxidant enzymes may lead to the loss of animal appetite due to stress (38). However, the anorexia brought on by heavy metal consumption might explain why those who are continuously exposed to lead lose weight. (39). These effects significantly changed when the Lead Acetate was combined with L-Carnitine during both intervals of combination treatment. In contrast, the combination of Lead Acetate with Nano L-carnitine would significantly improve weight gain during the 60 days of treatment. It has been established that exogenous L-carnitine is a free radical scavenger and protects cells against oxygen radicals, which may explain the improvement in final body weight and partial improvement in weight growth caused by therapy with L-Carnitine and Nano L-carnitine. (40). L-carnitine improves lipid metabolism and insulin-like growth factors (IGFs) and IGF binding proteins in rats; LC effect on Leptin, a hormone released by adipose tissue, regulates food intake and body weight through interactions with hypothalamic neural circuits depending on the levels of triglyceride reserves, and hence plays an important role in energy balance management. (41). According to research, L.C. acts as a free radical scavenger and enhances antioxidant enzymes like glutathione peroxidase and vitamins A and E to protect cells from oxidative stress-induced harm. (42). As a result, this research was carried out to evaluate the potential protective function of L.C. against the harmful effects of lead on body weight.

Under the impact of lead, oxidative stress develops as a result of two parallel pathways: first, the production of reactive oxygen species (ROS), such as hydroperoxides (HO2•), singlet oxygen, and hydrogen peroxide (H2O2), and second, the depletion of antioxidant reserves (43). In terms of ROS susceptibility, lipids are among the most vulnerable biological substances. Unsaturated fatty acids, which are found in cellular membranes, tissues, and blood, are particularly vulnerable to ROS attack. The rise in plasma cholesterol levels might be attributable to increased cholesterol production in the liver, or it could indicate liver damage caused by lead acetate affecting the permeability of the liver cell membrane (44). Furthermore, an elevation in plasma total cholesterol may be due to a blockage of the hepatic bile ducts, resulting in a decrease or stoppage of bile secretion to the duodenum (45). An impairment of the lipase enzyme activity of both hepatic triglycerides and Plasma lipoproteins has been blamed for the rise in Plasma triglycerides (46). The liver and intestine cells produce the majority of HDL-C. It helps remove cholesterol from tissues and transports it back to the liver to be removed as bile acids (47).

Nano L-Carnitine would significantly improve lipids profile decrease in levels TG,TC,LDH, and VLDL and increase HDLc level are petter effect in male rats.

L-carnitine is a small water-soluble quaternary amine that plays a crucial role in lipid catabolism by acting as a necessary cofactor for transporting long-chain fatty acids through the inner mitochondrial membrane as L-carnitine esters (48). A recent study reveals that carnitines are important regulators of glucose metabolism and their involvement in fatty acid oxidation (49). Several investigations on carnitine metabolism have shown lower carnitine levels in the plasma and liver. (50).

The results of oxidative stress biomarkers revealed a significant decrease in antioxidant enzyme activities superoxide dismutase (SOD) and nitric oxide (NO) in rats given lead acetate for 30 days compared to the control group table (1).

ROS formation of ROS includes different pathways including hydroperoxides, singlet oxygen and hydrogen peroxides, resulting in cellular dysfunction. These results are in agreement with (51,52,53). Lead exposure causes intense lipid peroxidation, which damages the mitochondrial and cytoplasmic membranes, producing severe tissue oxidative damage and releasing lipid hydroperoxides into the blood (54,55).

Antioxidant enzymes can protect cellular compounds against damage induced by free radicals. Nitric oxide (NO), superoxide dismutases (SOD), and glutathione peroxidases (GPx) are essential antioxidant enzymes (56). The present study shows that l-carnitine therapy improves low plasma levels SOD, and NO, these results are compatible with (57,58). By activating superoxide O2 dismutation events, SOD protects oxygen-metabolizing cells from the adverse effects of free radicals (59). SOD degrades superoxide radicals by generating hydrogen peroxide, which may be removed by catalase or glutathione peroxidase (60). Different NO synthases (NOS) produce nitric oxide (NO): endothelium NOS, neuronal NOS, and inducible NOS. In a healthy kidney, nitric oxide is essential for regulating tissue damage and renal blood flow (61). L-carnitine therapy dramatically increased NO levels in the current research, most likely due to endothelial cells. This might lessen the severity of ischemia in the renal tissue (62). These effects decreased significantly when the administration of lead acetate with L-carnitine, while these effects declined to non-significant levels when compared with the control group in groups treated with lead acetate with Nano L-Carnitine in 60 days.

The Histopathological examination of sections in cerebrum cortex of Lead Acetate treated rats. Showed Alzheimer's type II astrocytes, characterized by double nuclei surrounded by clear space. Showed intracellular and extracellular vacuoles showed degeneration and necrosis of cortical neurons. The findings agreed with those of (63,64,65,66). When blood Lead Acetate levels rise, the blood-brain barrier is permeable to lead acetate, as indicated by the accumulation of lead acetate in the brain (67).

It is postulated that lead concentration increased in lead-exposed animals in the cerebellum and cerebral cortex. Moreover, long-term low-level occupational exposure to lead has been a risk factor for some neurodegenerative diseases such as Parkinson's and Alzheimer's. (68).

L-Carnitine and Nano L-Carnitine are administered concurrently with lead acetate for the LC+ lead group, and Nano LC+ lead groups, respectively. They have caused significantly improved in the cells and typical arrangement of the cerebrum and cerebellum layers.

In models of adult, neonatal, and brain damage, including hypoxia-ischemia and traumatic brain injury, Alzheimer's disease, and disorders causing central or peripheral nervous system injury, L-carnitine improves energy status, decreases oxidative stress, and avoids cell death. Apart from that (69). L-carnitine has a strong neuroprotective impact when given immediately after reperfusion from acute global cerebral ischemia. By (70).

L-carnitine supplementation may help prevent tissue shortage in this element and prevent oxidative damage. capacity L- carnitine to pass the blood-brain barrier suggests that it may potentially help reduce neurological damage caused by oxidative toxicity. (71)

7. CONCLUSION

lead acetate has to harmful effect on the body systems, especially on the central nervous system; Biogenic L-Carnitine Nanoparticles (LC-NPs) may improve body weight and decrease lipid profile except for HDL is increased. These results may reflect that LCNPs could be beneficial as a complementary agent in treatment against lead acetate toxicity.

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