International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 14s,2025
https://theaspd.com/index.php

Ameliorative Effect Of Spirulina In Mice Experimentally Exposed To Water Pollution With These Heavy Metals. (Lead And Cadmium)

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Abstracts
This study aimed to investigate the possible advantages of spirulina for mice subjected to heavy metal-contaminated water. Twenty mice were administered a dosage of 0.005 mg/L of cadmium chloride, while another twenty mice received the identical cadmium dosage along with a 3.0 g/kg spirulina suspension orally via gavage every five days, all while adhering to a standard diet. The results indicated that cadmium induced considerable liver damage in the mice, evidenced by necrosis, inflammation, and fatty alterations, while spirulina treatment markedly mitigated these histological abnormalities, safeguarding the integrity of the liver tissue.
Keywords/ *lead, cadmium. Pollution, Spirulina*

INTRODUCTION

The concentration of heavy metals in the environment has risen recently (Valverde et al., 2000). Mining and smelting activities produce two significant heavy metals into the environment: (Du *et al.,* 2020). Water may include cadmium, posing risks to both humans and animals (Srivastava et al., 2024). **(Hashem and Abdaljaleel, 2024).** The ingestion of some foods can elevate the tissue levels of CdCl2, a compound present in soil and vegetation. Cadmium exposure results in hepatotoxicity from acute therapy, characterized by necrosis, apoptosis, peliosis, and inflammatory infiltration (Kyriakou et al., 2013). **Jassim *et al.,*2021)** cadmium accumulates in the liver, resulting in harm and disruption of normal organ function. Research has examined spirulina's hepatoprotective properties; its bioactive compounds may mitigate heavy metal-induced hepatic damage and facilitate the body's detoxification mechanisms. **Al Sulivany and Associates, 2024** Cadmium (Cd2+) compounds, particularly CdCl2, are widely recognized for their potential toxicity to people and animals due to their rapid distribution in tissues **(Abdulqader, 2022) (Hameed, 2024).** Individuals utilize natural herbs daily owing to their numerous biological and pharmacological qualities (Hosseini Mehr, 2014). The potential chelation activity, or the capacity to bind heavy metals and facilitate their excretion from the body, is a primary reason why natural products such as spirulina are beneficial against heavy metals. **Abu-Almaaly et al. (2023).**

Research indicates that compounds in spirulina, including amino acids, polysaccharides, and certain peptides, may facilitate the binding of heavy metals such as lead and cadmium, so mitigating their toxicity. This process may boost the body's ability to excrete these metals via faces or urine. **Mendes et al. (2024)**. This work aims to investigate the experimental evidence of SP's protective effects against cadmium-induced hepatic damage and the public health implications of heavy metal exposure in the livers of experimental mice.

MATERIALS AND METHODS

Animals

Sixty mice, each weighing between 25 and 30 grams, were randomly allocated into three groups, comprising 20 mice per group, for the present investigation. The mice were housed in plastic containers measuring 20 × 50 × 75 cm at a designated site within the University of Baghdad's Department of Public Health, College of Veterinary Medicine. Following a week of

acclimatization, tap water and commercial feed pellets, the standard diet for mice, were provided ad libitum. Housing conditions in climate-controlled chambers with a 12-hour light/dark cycle were sustained at 20–25 °C. A ventilator was consistently employed to alter the air in the rooms, and the waste from the containers was replenished daily.

Experimental Design

Three groups of sixty mice, each including twenty mice, are established

Group A: (negative control): Twenty healthy mice were administered simply distilled water .orally and were not subjected to Cd infection

Group B : receives a standard meal alongside a hazardous dosage of cadmium chloride (0.005 .mg/L) delivered by stomach tube every two days for a duration of three months

Group C: of the ailing mice, subjected to a hazardous concentration of 0.005 mg/L of cadmium chloride, received a spirulina suspension by stomach tube every five days at a dosage of 3.0 g/kg body weight, in conjunction with a standard diet.

Blood Serum Samples.

It was utilized for the occasion three months after the administration of the medication. Chloroform was employed to anesthetize all the mice. Mice were directly poked to acquire blood samples, subsequently transferred to sterile, dry, and clean gel tubes. The samples were allowed to clot at ambient temperature for a limited duration before being centrifuged for 15 minutes at 4000 rpm to separate the clear sera. A micropipette was utilized to transfer the sera to Eppendorf tubes, which were then stored in a deep freezer at -20°C until the completion of biochemical analysis.

Biochemical test

liver function tests (ALP, ALT, AST) were conducted using blood samples from mice.

Histopathological Pathological Examination:

Upon completion of the experiment, the animal was anesthetized via chloroform inhalation, followed by a comprehensive pathological examination. Following excision, a segment of the liver was placed in plastic containers containing 10% formalin. All groups engaged in the study (Abd-AlHassen, 2019). **(Jaber *et al,*2021).** Samples were initially desiccated utilizing various ethanol concentrations, subsequently embedded in paraffin wax, and sectioned to a thickness of 4 micrometers using a microtome. Subsequently, they were stained with hematoxylin and eosin for histological examination and microscopic evaluation (Suvarna et al., 2018). After the preparation of the tissues, each tissue block from Z. Goodarzi et al. was sectioned into 10 µm slices, which were subsequently stained with hematoxylin and eosin (H&E) dyes. Nuclear dilatation, diminished staining capacity, and pronounced cellular edema were seen as markers of cellular damage. One pathologist and one histologist randomly inspected each slide, focusing on five fields at a 200X magnification.

Dissection.

Table (1) the effect Cadmium concertation on liver enzyme in mice

Groups	ALP	ALT	AST
1 Control	55.35±2.93b	44.75±4.12c	93.55±3.99d
2 Cd	84.25±5.56a	77.50±3.53ab	263.25±10.91a
6 Cd+ spirulina	69.15±6.28b	59.40±5.31c	206.40±24.36ab
LSD	14.87	10.26	60.71

Table 1 illustrates the effect of therapies on liver enzymes. ALP levels were significantly elevated in the Cd-contaminated water (0.005 mg/l $P < 0.01$) compared to the control group animals. This results in elevated ALT levels in mice, signifying hepatocyte death and liver damage mediated by oxidative stress. Table 1 indicates that AST levels in mice exposed to cadmium in

water significantly elevated relative to the control group, corroborating the results of Goodarzi et al. (2020) and Khalaf et al. (2023). **(Ahmed and Mohammed, 2022)**. Serum AST levels significantly increased ($P < 0.01$) following three months of Cd treatment (0.005 mg/l, $P < 0.01$), indicating hepatic injury due to AST leakage from damaged hepatocytes. This finding corroborates the results of Liu et al. (2020). ALP levels were markedly elevated in mice exposed to Cd compared to the control group (Table 1). Increased serum ALP levels after three months of Cd therapy indicated potential liver damage. (Gharbi et al., 2022; Yang et al., 2021). Research indicates that spirulina positively influences oxidative stress in the liver induced by cadmium. In experimental mice, pretreatment with spirulina reduces increased liver enzyme levels induced by cadmium. Animals provided spirulina exhibited a statistically significant reduction ($P < 0.05$) compared to the saline-treated group. This result aligns with the findings of **(Mallamaci *et al.* 2024; Mustafa, 2020).**

Histopathological examination

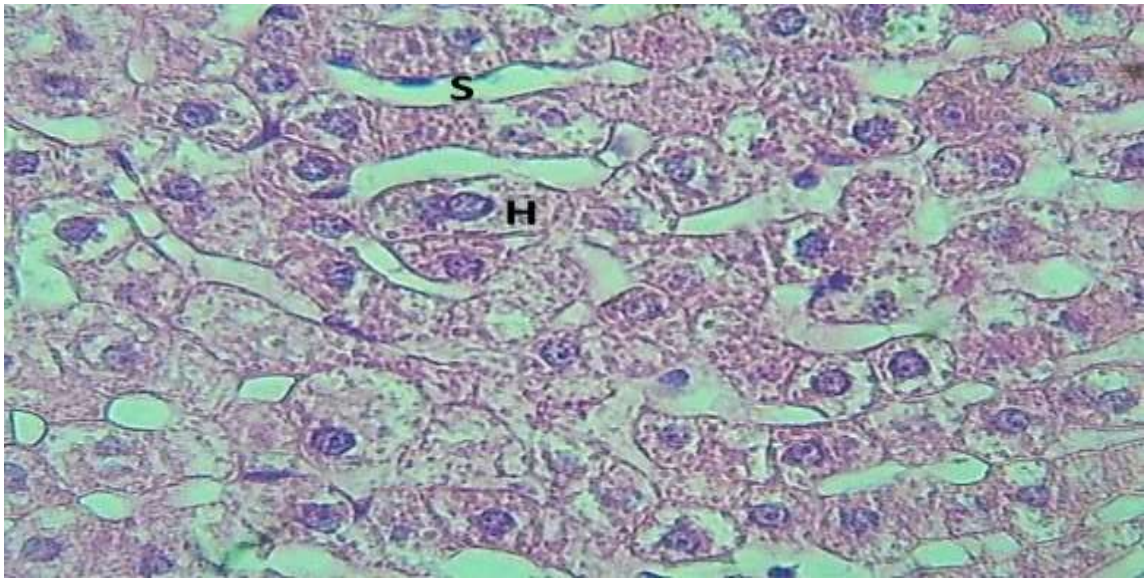

Figure 1: section of hepatic portal triad (Control negative) shows: normal sinusoid (S)& normal hepatocytes (H). H&E stain.400x.

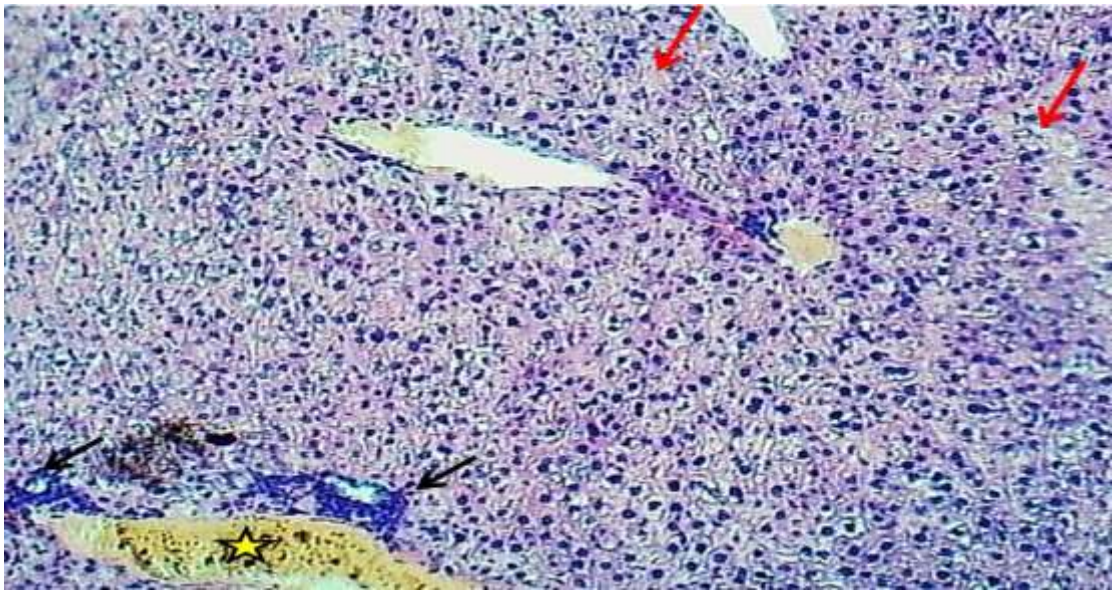

Figure 2: section of liver (Cd) shows: disarrangement of hepatic cords with vacular degeneration and necrosis (Red arrows), mild pre central vein aggregation of lymphocytes, portal pre vascular lymphocytic aggregation (Asterisks). H&E stain.100x.

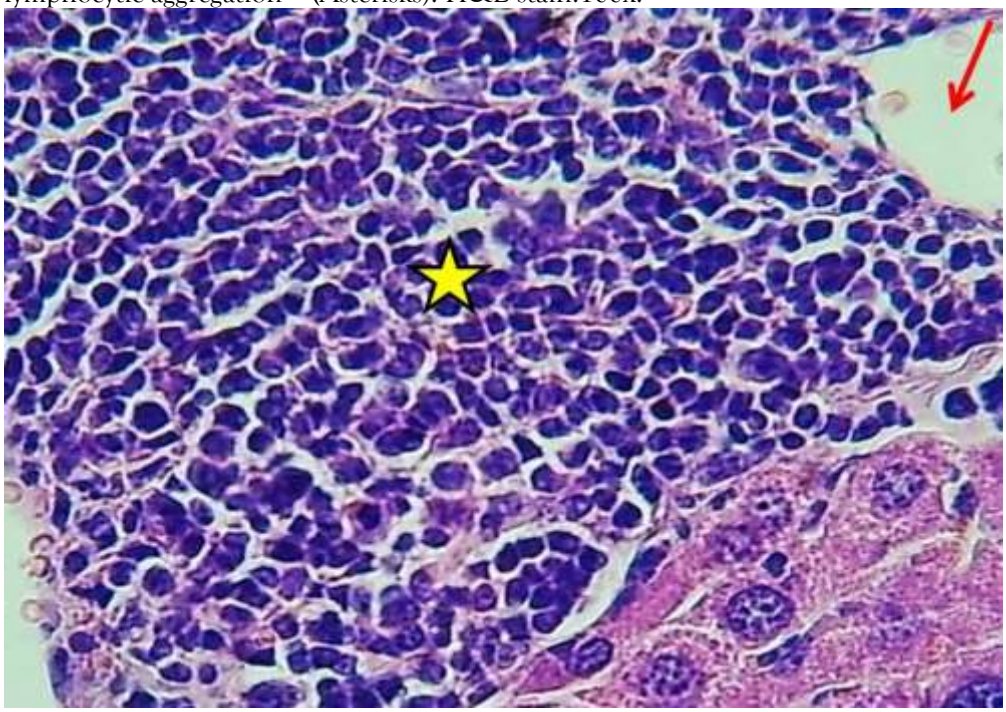

Figure 3: section of hepatic portal triad (Cd) shows: portal aggregation of mononuclear leukocytes (asterisk) & dilation of portal vein (Red arrow). H&E stain.400x.

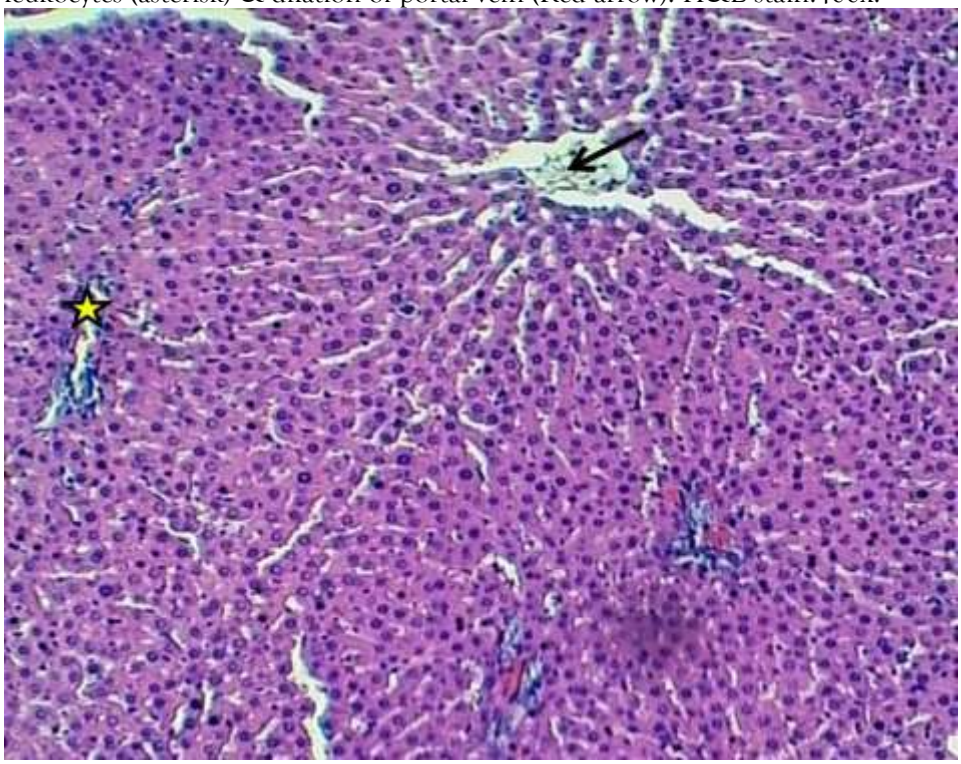

Figure 4: section of liver (Combination of cadmium +spirulina) shows: normal central vein (arrow) with mild sinusoidal congestion and normal portal triad (Asterisk). H&E stain.100x.

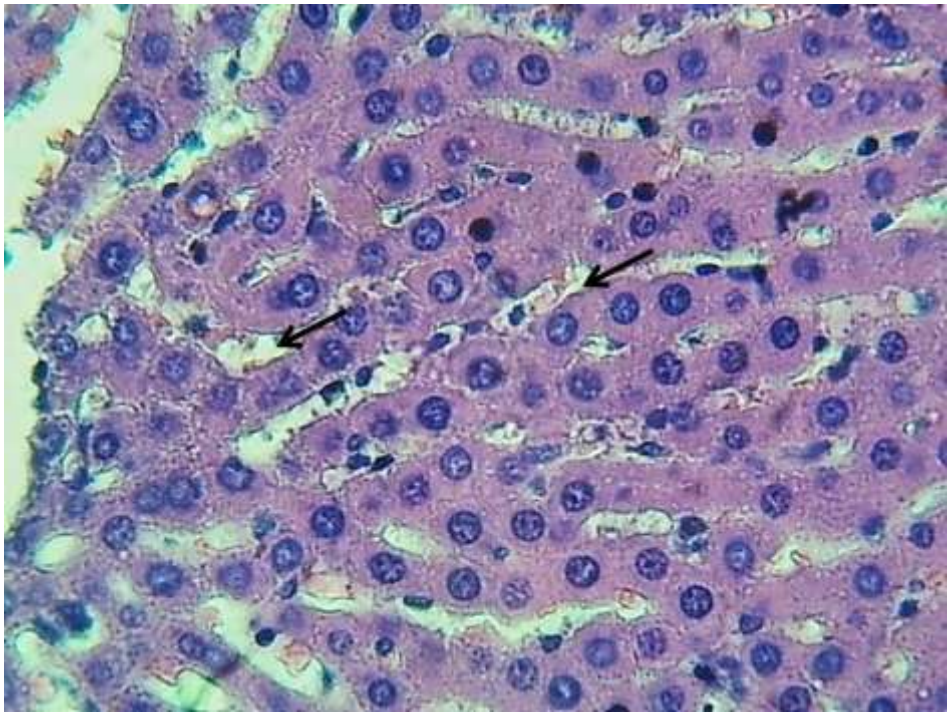

Figure 5: section of liver (Combination of cadmium +spirulina) shows: normal central vein with mild sinusoidal congestion (Arrows) and normal hepatocytes. H&E stain.400x.

Figure 1 illustrates that the livers of control mice had normal hepatocytes and sinusoids (S), signifying typical cytoarchitecture. Mice administered in the cadmium for three months had hepatic tissue damage in their livers. The histological examination of the hepatic lobules revealed considerable disorganization of the hepatic cords, characterized by vascular degeneration and hepatocyte necrosis (figure 2). A slight accumulation of mononuclear leukocytes, predominantly lymphocytes, and mild congestion were noted in the central vein (fig. 3). This aligns with the studies conducted by Liu et al. (2020). The histological examination of the liver post-CdCl2 treatment revealed a normal central vein, a normal portal triad, and an orderly configuration of the hepatic cords, accompanied by minor sinusoidal congestion (fig.4). Magnified images revealed a characteristic morphology of hepatocytes with minimal sinusoidal congestion (fig. 5). This result aligns with the findings of Sakr et al. (2015). (Rady et al., 2023)

CONCLUSION

An experimental investigation indicates that cadmium significantly impacts liver function. Spirulina is a nutritional supplement that significantly diminishes heavy metal contamination in water, as evidenced by the improved outcomes observed in the cadmium group administered spirulina.

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