The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 5, 2026

HEPATOPROTECTIVE EFFECTS OF BIOLOGICALLY SYNTHESIZED ZINC OXIDE AND SILVER NANOPARTICLES AGAINST CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN *RATTUS NORVEGICUS*

Arham Dar[1], Hamza Noman[2], Abdullah Ghulam Nabi[3], Anam Amir[4], Nayab Munawar[5], Hafiza Amna Ahmad[6], Rahish[7], Usman[8], Mehreen Fatima[*9]

[1,3,6,7, 8,*9]*Department of Life Sciences, University of Management and Technology, Lahore, Pakistan*
[2]*Department of Biological Sciences, The Superior University, Lahore, Pakistan*
[5]*Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan*

[*8]mehreen.fatima@umt.edu.pk

DOI: https://doi.org/10.5281/zenodo.20307079

Keywords
Zinc oxide nanoparticles, Silver nanoparticles, Liver protection, Carbon tetrachloride, Nanomedicine.

Article History
Received: 16 March 2026
Accepted: 26 April 2026
Published: 20 May 2026

Corresponding Author: *
Mehreen Fatima

Abstract

Background: *Experimental models have widely employed the use of Carbon tetrachloride (CCl4) as a hepatotoxic agent in experimental models for the investigation of liver injury and evaluate its potential therapeutic interventions for its treatment. Nanoparticles, particularly zinc oxide (ZnO) nanoparticles, have received increasing attention for their biomedical applications.*

Objectives: *This study employed the use of biologically synthesized ZnO nanoparticles and evaluated them for their hepatoprotective effects on liver injury induced by CCl_4.*

Method: *Experimental group was subjected to CCl_4 treatment alone or in combination with the synthesized ZnO for 14 days.*

Main outcome measure: *This treatment was followed by biochemical analysis, gross anatomical examination, and histopathological evaluation of liver tissue.*

Results: *Rats treated with CCl_4 alone showed marked liver injury, reduced body weight, and altered feeding behavior. In contrast, rats subjected to CCl_4 in combination with nanoparticles showed improved biochemical parameters, body weight was sustained, and reduced signs of histological damage were recorded.*

Conclusion: *These findings demonstrate that ZnO nanoparticles exhibit protective effects against chemically induced liver injury. Further studies with larger sample sizes and mechanistic investigations are required to confirm their therapeutic potential and explore possible applications in nanomedicine.*

1. INTRODUCTION

Cancer is a complex group of diseases characterized by uncontrolled cell division and the potential to form tumors [1]. These mutations in DNA can result from various factors, leading to both benign and malignant tumors, as well as non-tumor-related cancers such as lymphomas, leukemia, and myeloma. Cancer progresses through four stages: potential pre-cancerous cell abnormalities, localized cancer cells, cancer spread to nearby tissues and lymph nodes, and metastatic cancer affecting distant body parts [2]. Metastatic cancer occurs when cancerous cells travel from the primary site to other areas of the body through the bloodstream or lymphatic system [3]. Such dissemination is determined by the type of cancer and may cause secondary, rather than

primary tumors [4]. Cellular functions are governed by genes and when these genes are mutated, they may cause cancer. The mutations may be obtained through external sources such as radiation, UV light or tobacco or they may be inherited via germ line mutations [5].

Liver is an important organ within the body, actively involved in metabolism, blood filtration, bile production and important proteins formation [6]. The liver cirrhosis is a serious liver disease characterized by the formation of scar tissue and the impairment of liver functions due to alcohol abuse, virus infection or genetic components [7].

Recent years have marked the emergence of nanotechnology as a promising field for biomedical research. They have served as vital tools for diagnosis, drug delivery and therapeutic intervention. Nanoparticles are characterized by unique physiochemical properties such as high surface area, tunable surface chemistry, and enhanced bioavailability. These traits allow nanoparticle to have better adaptation and interaction with biological environment. Hence, nanoparticles based strategies open frontiers in targeted therapies and reduced systemic toxicity [8].

Among various nanomaterials, biologically produced Zinc oxide nanoparticles have been specifically identified for their biocompatibility, antimicrobial activity, antioxidant potential, and emerging therapeutic applications. ZnO nanoparticles have demonstrated antioxidant, anti-inflammatory, and cytoprotective properties making them potential candidates for evaluation as an anti-cancer and anti-bacterial agents [9]. In cancer treatment they possess specific cancer cell cytotoxic activity while minimizing harm to healthy cells [10]. However, concerns remain regarding their potential toxicity, as they may disrupt cell cycle, generate stress, cause DNA damage and induce apoptosis. This emphasizes the need for systematic in vivo evaluation of their safety and biological effects [11].

The present study aimed to investigate the hepatoprotective effects of biologically synthesized ZnO nanoparticles against CCl_4-induced liver damage in *Rattus norvegicus*. By combining biochemical assays, gross anatomical examination, and histopathological analysis, this work seeks to provide insight into the protective potential of these nanoparticles and their possible implications in the development of nanomedicine-based therapeutic strategies [12].

2. MATERIALS AND METHODS

Animal Model

The study employed common laboratory rats, specifically albino purebred *Rattus Norvegicus*. Three rats of similar weight were divided into three groups: Normal or healthy rats, rats with carbon tetrachloride-induced disease, and rats subjected to carbon tetrachloride's toxic effects along with zinc oxide nanoparticles.

Zinc Oxide Nanoparticles used in the study were biologically synthesized and generously provided by Institute of Molecular Biology and Biotechnology, The University of Lahore.

The carbon tetrachloride used was 99.5% anhydrous sourced from Sigma Aldrich in Steinheim am Albuch, Germany with catalogue number 32215 [13]. For administration, equal volumes of carbon tetrachloride and olive oil were mixed in a 1:1 ratio and vortexed for 20 minutes to ensure uniform mixing. One of the rats received CCl4 and ZnO nanoparticles for a period of fourteen days based on its weight. Another rat received CCl4 for the duration taking into account its weight. The control group did not receive any treatment while experimental groups 2 and 3 were given CCl4 in a 1;1 ratio along, with nanoparticles.

Phosphate-Buffered Saline (PBS) Sigma-Aldrich's PBS was used to maintain a continuous pH level, with catalogue number P5493 from Bavaria, Germany [14]. Formalin A 10% neutral buffer solution of formalin was utilized to preserve liver samples for histopathology after dissection. It was obtained from Bavaria, Germany, with catalogue number HT501128 [15]. Olive Oil Olive oil, sourced from Sigma-Aldrich with catalogue number O1514 in St. Quentin Fallavier, France, was employed for diluting carbon tetrachloride at a 1:1 ratio [16].

2.1 Preparation of Zinc Oxide Nanoparticles (ZnO)

Zinc oxide nanoparticles were biologically synthesized in powdered form. There were then subjected to in vitro studies that verified their successful formation. These nanoparticles were accurately weighed and suspended in a 1.5ml microcentrifuge tube containing 1ml of PBS

solution. Subsequently, they were sonicated for approximately two hours and vortexed for twenty minutes. For daily dosages, the nanoparticles underwent one hour of sonication and ten minutes of vertexing [17]. During the 14 days experiment we maintained a room temperature of 36.5^{0}C. The subjects were provided with a protein and fiber diet, for poultry along with an appropriate amount of water. We also observed changes in their eating habits based on the dosage of CCl4 and zinc oxide nanoparticles. Interestingly both the body weight and fur color of the rats were affected by these substances. In particular rats treated with zinc oxide nanoparticles experienced a change in fur color. This change was characterized with a paler fur in comparision to rats not administered with substances.

To determine the dosage, the following calculations were made:
Maintained stock: 5mg/kg/day
For a 150g rat: 0.75mg/150g/day
For 14 days: 0.75 x 14 = 11.25mg/14 days
15mg of ZnO nanoparticle was dissolved in 1ml of PBS solution, equaling 15µg in 1µl.
We required 750µg for one rat, so 15µg was multiplied by 50, resulting in 750µg.
The final concentration administered to a 150g rat was 50µl of ZnO nanoparticle

2.2 Preparation of Carbon Tetrachloride (CCl4)

Carbon tetrachloride was prepared in a 50ml falcon at a 1:1 concentration with 100% pure olive oil. It's important to handle CCl4 with care, as it is a toxic substance that can cause skin irritation (Table 1) [18].

For dosing calculations:
CCl4 for each rat was 375µl/kg, an optimized concentration.
For a 150g rat: 375/1000 x 150 = 56.25µl
56.25µl of CCl4 was administered to the rat.
CCl4 group rats had a weight of 164g on day zero, so they received 61.5µl on that day.
Zinc Oxide + CCl4 rats had a weight of 142g on day zero, and they received 53.25µl of CCl4.

Table 1: Catalogue number with kit size for order information

1 2601 99 10 021 R_1	5 x 20 mL + R_2	1 x	25 mL
1 2601 99 10 026 R_1	5 x 80 mL + R_2	1 x	100 mL
1 2601 99 10 023 R_1	1 x 800 mL + R_2	1 x	200 mL
1 2601 99 10 704 R_1	8 x 50 mL + R_2	8 x	12.5 mL
1 2601 99 10 917 R_1	8 x 60 mL + R_2	8 x	15 mL
1 2601 99 90 314 R_1	10 x 20 mL + R_2	2 x	30 mL

2.3 Dosage

One group of rats received CCl4 and ZnO nanoparticles for fourteen days, while another group received CCl4 only, serving as a comparison. A control group of rats received a standard diet and water without any injections, providing a reference for normal rat conditions (Table 2) [19].

Table 2: Total study of Rats divided into three groups on the basis of treatment

Sr. No.	Animal and group type	Treatment given to animal
1	One rat only and control group.	No treatment was given to this animal.
2	One rat only and diseased group.	CCl_4 in 1:1 with olive oil was administered to this rat.
3	One rat only and this is a combination group.	CCl_4 in 1:1 with olive oil and zinc oxide nanoparticles were administered to this rat.

2.4 Sacrifice

After 14 days, all rats were dissected, and their liver organs and blood samples were collected. Liver specimens were fixed in formalin for histopathology, and blood samples were subjected to laboratory tests [20].

2.5 Aspartate Transaminase (AST)

The reagent kit that was used here was ASAT (GOT) FS*. It's an optimized UV test according to International Federation of clinical and laboratory medicine.

2.6 Alanine Aminotransferases (ALT)

Kinetic test was performed for this test according to International Federation of clinical and laboratory medicine.

3. RESULTS

3.1 Weight of Rats

The weight of an organism is contingent upon a multitude of intrinsic factors, and the subject of this investigation centers on the pivotal influence of liver cirrhosis induced by the administration of carbon tetrachloride (CCl4). Over the course of a fortnight, an observable alteration in the body weight of the test subjects, namely rats, has been meticulously documented. This study delves into the complex interplay between liver cirrhosis and its subsequent impact on the body weight of rats. The pivotal factor under scrutiny is the induction of liver cirrhosis through the administration of carbon tetrachloride (CCl4), a potent hepatotoxin widely employed in experimental settings to simulate liver damage. The idea of using this chemical compound is to induce a condition similar to that of the human liver cirrhosis which is usually marked by a high degree of fibrosis and interference of normal liver functions.

The test group was rats, which were used as the experimental organism due to their physiological similarity with human beings with respect to liver functionality and the possibility to conduct test experiments under laboratory conditions. The researchers were very attentive to observe the changes in body weight of these rats during a particular period of time (14 days). The choice of this time window is critical as it allows for the examination of short-term effects, and it aligns with the timeframe within which liver cirrhosis often exhibits significant changes in the clinical context.

3.2 Liver Weights of Rats

Liver weights of rats are also affected by CCl4 and combination group. The liver collected for chemical analysis. Blue line shows control group. Red line shows diseased with CCl4, Grey line shows group which got combination of CCl4 and Zinc nanoparticles [21]. The liver weight of rat was administered with CCl4 decreased significantly as compared to the rat that was administered with CCl4 and Zinc oxide nanoparticle; furthermore, the rat that was present in control also have a higher weight than the rat who was induced with CCl4 Figure 1. [22].

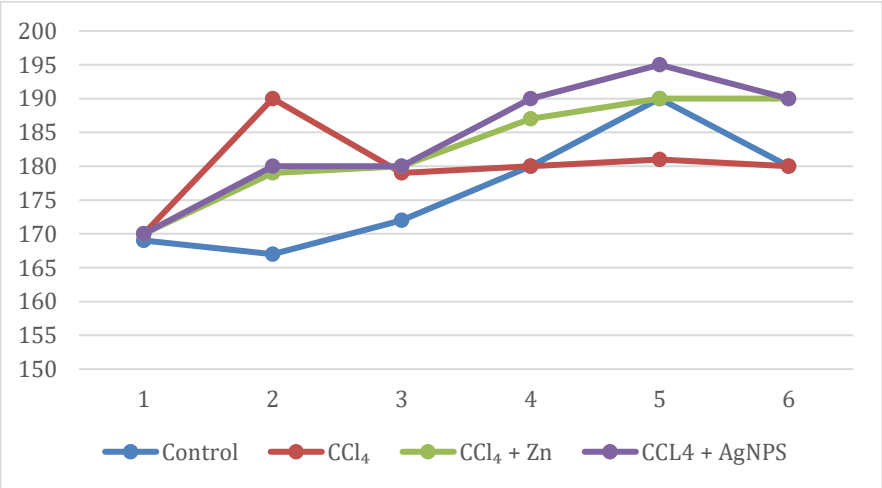


Figure 1: The weight of rat dropped substantially as compared to combination or control rat

3.3 Lactate Dehydrogenase Enzyme

LDH has significant increase in CCl4 administered rat as compared to the combination group, which is an indication of liver damage. It is an indication of organ damage.

3.4 Alanine Transferase Enzyme

Liver injury induced by the administration of carbon tetrachloride (CCl4) is a crucial aspect of this study. The graph depicting alanine aminotransferase (ALT) levels reveals a comparative analysis between the combination group, which includes the introduction of CCl4, and the CCl4-only group. ALT is an enzyme that is mainly located in the liver and its concentration is a pointer of liver damage. The comparison of the ALT levels of these groups will help to gain essential information about the degree of liver damage inflicted by CCl4 and how the combination of factors, potentially therapeutic interventions or other treatment, can affect liver health. The information is crucial in the perception of the effects of CCl4-induced liver damage, as well as the possible approaches to reduce the damage or enhance liver repair.

3.5 Aspartate Aminotransferase

AST or Aspartate Aminotransferase enzyme is a biomarker to determine liver condition and higher increase in levels is linked to liver damage. In the provided graph, a comparison between the combination group, involving the introduction of CCl4 and zinc nanoparticles, and the CCl4-only group is made. While the levels of AST appear to be higher in the combination group, the difference observed does not significantly indicate liver damage due to zinc nanoparticles. This suggests that the introduction of zinc nanoparticles, in the context of this study, does not appear to exacerbate liver damage as measured by AST levels when compared to the CCl4 group. However, further analysis and investigation may be required to fully understand the implications of zinc nanoparticles on liver health and to assess their overall safety in this experimental context.

3.6 Alkaline Phosphatase

It is known as ALP test. High levels of ALP show damage to the liver. Combination group indicates high value but it's normal in blood stream. Moreover, there is no sign of cirrhosis in liver shown in Figure 2.

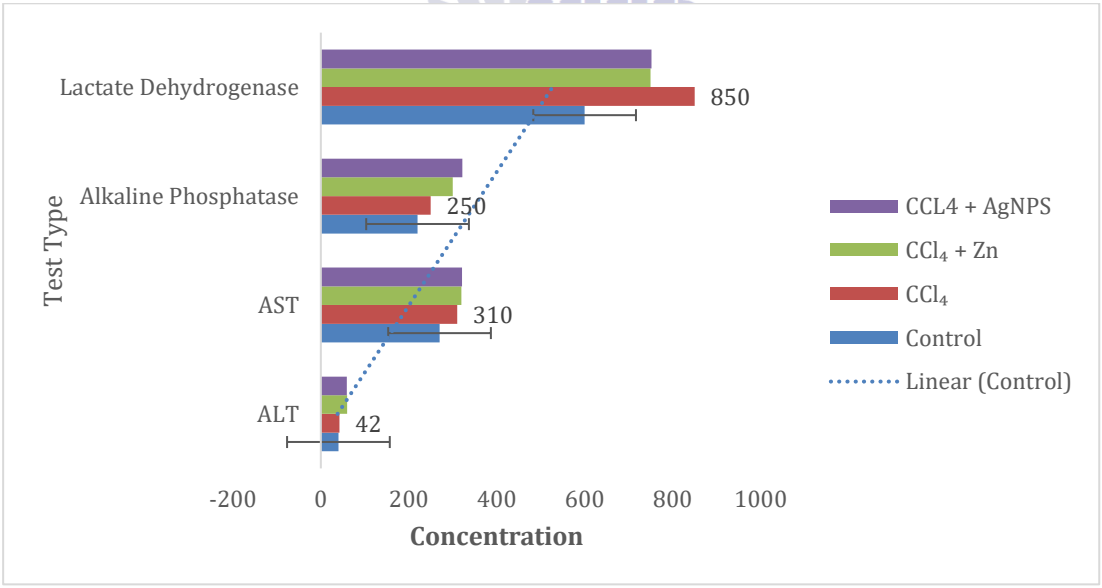


Figure 2: In this figure LDH, Alkaline Phosphatase, AST and ALT values with respect to the rats are given, Rat were administered with CCl4 has relatively higher values as compared to the control, or the rat that was administered with CCl4, Zinc oxide nanoparticles.

3.7 Total Protein

Total protein test also shows liver damage or high protein in blood causes liver cirrhosis. This graph shows normal serum protein level in blood.

3.8 Bilirubin Total

The provided figure displays normal bilirubin values within red blood cells. Elevated bilirubin levels, as indicated, can be a sign of liver cirrhosis and various other liver-related diseases. Bilirubin, a product of red blood cell breakdown, is processed by the liver, and its levels within the normal range signify a healthy liver. However, heightened bilirubin levels signal potential liver dysfunction, making this figure a valuable diagnostic tool for recognizing liver-related health issues, including the serious condition of liver cirrhosis.

3.9 A/G Ratio

Albumin and Globulin ratio has been shown. High level of A/G ratio indicates damage taking place inside the liver. over all there is less damage in the liver in chemical analysis seen in Figure 3.

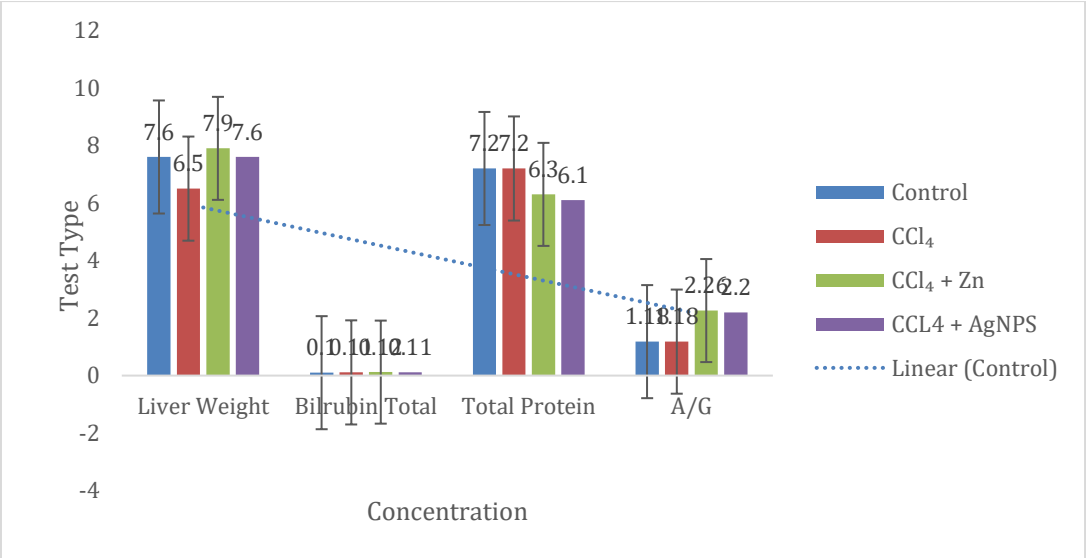
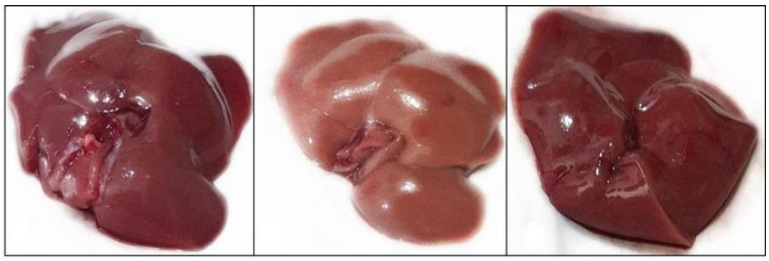


Figure 3: This figure shows the bilirubin total, Total Protein and A/G inside the liver, ratio has been shown high level of A/G ratio

It indicates damage taking place inside the liver. On the y-axis values of A/G ratio are present and on the x-axis rats are present. Rat number one is control; rat number two is the one which was administered with CCl4 and Zinc oxide nanoparticles. Rat number three was administered with CCl4, and shows relatively higher A/G value as compare to other rats.

3.10 Liver Anatomy

(A): Liver of rat administered with zinc oxide nanoparticle and carbon tetrachloride. The color of liver was of slightly dark color. There was no other anomaly present on the liver [23]. Piece of liver was taken and it was dipped inside the formalin for the histopathology analysis [24]. All other vital organs were also free from any type of anomaly.
(B): Liver of rat administered with carbon tetrachloride. The color of this liver had a pink due to it. There were white spots present on some parts of this liver. It is an indication of damage to the liver; there was not any further anomaly found on this liver [25]. A dark color spot can also be seen on one of the lobes which was quite odious.
(C): This is the liver of a rat that was present in control. There was no anomaly found on this liver. The liver of this rat was in normal color with no signs of damage at any place. Rests of organs were clear from any type of anomaly is shown in Figure 4.



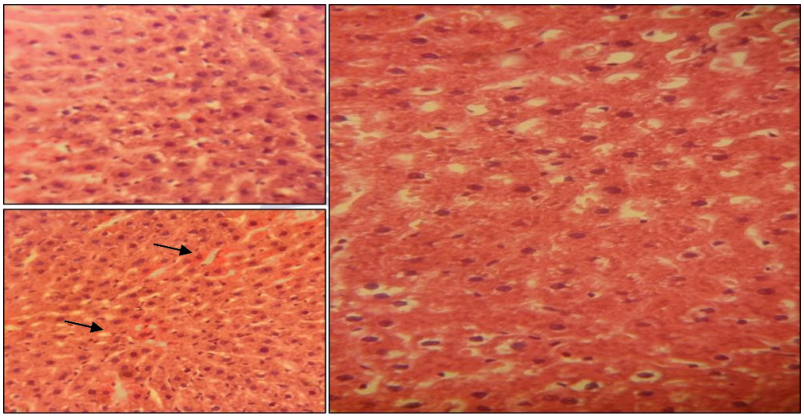
A B C

Figure 4: Normal and diseased liver of rat compared with combination group

3.11 Liver Histopathology

(A): This is a high-power magnification. It is a histopathology slide of liver cells control and carbon tetra chloride induced liver of rats. Due to the carbon tetra chloride there is change in structure.

(B): There is no architectural difference visible here. Cells are in normal shape and size. There is no collective mass, or anomaly is present here. These cells look normal in this slide, and macrophages are also present in normal quantity shown in Figure 5 with catalogue shown in (Table 3). The effect of carbon tetrachloride with biologically synthesized Zinc oxide nanoparticle on *Rattus Norvegicus* was a main theme of whole research. The results obtained in the end are pretty much conclusive, and can be used for the greater good.



A B

Figure 5: This figure shows ultra-structural hepatic changes in CCl4 treated and combination in rat liver. The following histopathological slides were observed at Observed at 100X power.

3.12 Total Bilirubin

The test here was performed by using 2, 4 dichloroaniline (DCA).

Table 3: It shows catalogue number with reagents for order information.

Cat. No.	41131	41132	41133
Reagents	9 x 50 mL	90 x 50 mL	40 x 50 mL

3.13 Alkaline Phosphatase

The test that was conducted here was photometric test optimized standard method according to the German Society of Clinical Chemistry (DGKC) seen in (Table 4 and Table 5).

Table 4: It shows catalogue number and reagents quantity for order information.

1 0401 99 10 021	R_1	5 x 20 mL + R_2	1 x	25 mL
1 0401 99 10 026	R_1	5 x 80 mL + R_2	1 x	100 mL

1 0401 99 10 023	R_1	1 x 800 mL + R_2	1 x	200 mL
1 0401 99 10 704	R_1	8 x 50 mL + R_2	8 x	12.5 mL
1 0401 99 10 930	R_1	4 x 20 mL + R_2	2 x	10 mL
1 0401 99 90 314	R_1	10 x 20 mL + R_2	2 x	30 mL

Table 5: It shows catalogue number and reagent amount for order information

1 0811 99 10 021	R_1	5 x 20 mL + R_2	1 x	25 mL
1 0811 99 10 026	R_1	5 x 80 mL + R_2	1 x	100 mL
1 0811 99 10 023	R_1	1 x 800 mL + R_2	1 x	200 mL
1 0811 99 10 704	R_1	8 x 50 mL + R_2	8 x	12.5 mL
1 0811 99 10 917	R_1	8 x 60 mL + R_2	8 x	15 mL
1 0811 99 10 930	R_1	4 x 20 mL + R_2	2 x	10 mL
1 0811 99 90 314	R_1	10 x 20 mL + R_2	2 x	30 mL

4. DISCUSSION

Liver cirrhosis is an irreversible condition characterized by the continuous scarring and regeneration of liver tissues [26]. It is often caused by factors such as viral infections and alcohol consumption and can progress to liver cancer, ultimately resulting in death [27]. This study explores the effectiveness of zinc nanoparticles, particularly zinc oxide nanoparticles, in treating liver cirrhosis in rats [28]. Zinc oxide nanoparticles have received much interest in nanotechnology because of their variety of application [29]. Nanotechnology especially the application of zinc nanoparticles presents a better method of cancer treatment compared to alternative methods as it can selectively target only the cancer cells causing the cancer without attacking normal cells [30]. This study aims at analyzing the possible advantages of zinc nanoparticles in the treatment of liver cirrhosis [31]. It has been established that Zinc oxide nanoparticles have low toxicity levels in healthy cells, and hence it can be used in a number of medical applications [32]. The findings in this paper prove non-toxicity of normal cells and the efficacy of zinc nanoparticles in the treatment of liver cirrhosis [33]. These results indicate that zinc nanoparticles may become a drug to prevent cirrhosis in the future [34]. The liver fibrosis can be caused by chronic liver diseases that in most cases are brought about by factors such as abuse of alcohol and obesity [35]. The condition is reversible but if not treated, it can be transformed into irreversible liver cirrhosis. Zinc has a significant role in different metabolic processes in the body and deficiency of zinc may cause growth retardedness, neurological disturbances, lack of wound healing and metabolic disturbances [36]. Muscle cramps are commonly regarded as a consequence of liver cirrhosis, and zinc supplementation has been proven to positively affect the reduction of this condition [37]. Also, zinc helps in glucose metabolism that is hampered in cirrhotic patients and zinc is vital in insulin activity [38]. Zinc supplementation is beneficial in restoring liver functions and enhancing the metabolism of drugs, which makes it a possible remedy against liver cirrhosis [39].

Silver nanoparticles are advantageous due to their distinctive characteristics which make them the suitable particles to be used in the treatment of some kind of cancer. Through the use of silver nanoparticles, an example is when a cancer is found in close proximity to vital organs and it is not possible to remove it through surgery thus the use of silver nanoparticles can target the cancer cells only. This is able to increase the survival rates of the patient. Also, chemical therapy and radiation therapy can be enhanced by using silver nanoparticles. With the surface modification of silver nanoparticles with drugs or radiation used in chemotherapy, a precision targeting of cancer cells is achievable, which makes them more effective and the side effects of conventional methods of cancer treatment are minimized. Silver nanoparticles can be used in the treatment of cancer, but this remains in its preliminary phases, and a lot needs to be studied to realize their potential. Nevertheless, it is already possible to indicate that the silver nanoparticles can be used in the treatment of some cancers. As an example, a

research study by [36] established that it was possible to selectively kill cancer cells using gold nanoparticles. These researchers used gold nanoparticles which were coated with a protein which is known to adhere specifically to cancer cells. Their results showed that the nanoparticles were very effective in killing cancer cells without affecting the healthy ones. Different research revealed that it was possible to use silver nanoparticles to facilitate the efficacy of radiation therapy. The scientists targeted the cancer cells with the help of nanoparticles, and radiation therapy was applied. They discovered that the silver nanoparticles improved the performance of the radiation therapy thus leading to improved outcomes in the patients. A silver nanoparticle is not free of its adversities in its cancer treatment. The second major issue is to make sure the nanoparticles are specific to the cancer cells. It is possible to do this by applying a range of different techniques such as coating the nanoparticles with proteins or antibodies which are known to bind to the cancer cell in a specific manner. Moreover, the nanoparticles size is also significant. In case the nanoparticles are excessively large, they can be unable to penetrate the cells. In case they are too small they can be eliminated through the body before reaching the target cells. Nevertheless, there is still a potential in the research of gold nanoparticles with regard to cancer treatment. The peculiarities of silver nanoparticles. It has been proved that zinc deficiency has a connection with numerous diseases and it may influence the general body performance [40]. CCl4, in this study, induced cirrhosis in rats' liver and protective effects of zinc on the protecting liver fibrosis were evaluated [41]. The findings of the research have recommended that zinc acetate 10mg per kilogram of the body weight dose is the most effective dose in protecting the liver against CCl4-induced hepatotoxicity [42]. The toxicity of CCl4 was resisted by the zinc nanoparticles, especially the zinc oxide nanoparticles [43]. In vivo tests in healthy albino mice revealed that zinc oxide nanoparticles would provide protection against liver damage caused by CCl4 as assessed by liver functional tests [44]. The observations confirm the assumption that zinc nanoparticles would protect the liver against hepatotoxicity [45]. CCl4 treated rats (14 days) had visible liver damage, which was observed through gel electrophoresis or histopathology slides [46]. The research also entailed measurement of the levels of lactate dehydrogenase, which is a measure of tissue damage [47]. LDH levels were higher in rats subjected to CCl4 alone, which is a sign of liver damage. Conversely, both CCl4 and ZO nanoparticles treated rats recorded LDH levels within the normal range indicating protection of the liver [48]. Slides of histopathology showed arch changes in the livers of the CCl4 treated rats showing masses of cells and augmented cell size [49]. These are alarming and may portray the emergence of liver tumors. The protective effect of zinc oxide nanoparticles was also confirmed in a similar study against CCl4-induced hepatotoxicity [50]. Inflammation and cell accumulation were also detected which might be caused by the release of zinc ions that in turn induced the release of reactive oxygen species (ROS) and activation of P53 gene [51]. This gene is also connected to the process of causing apoptosis and it can be associated with the zinc oxide nanoparticles pathways [52]. Zinc oxide and silver nanoparticles have been found to selectively attack and kill cancer cells without attacking normal cells [53]. They are concentration-dependent and hence effective in targeted therapies. This study has found that the zinc and silver nanoparticles are selective in terms of attacking cancer cells and this property is applicable when combined with other chemotherapy agents [54].

To sum up, this study underscores the possible use of zinc oxide and silver nanoparticles in the treatment of liver cirrhosis. Their hepatotoxicity protection property and selectivity to cancer cells give them potential to develop as anti-cirrhosis drugs in the future. They are not toxic to other organ cells and this contributes even more to their safe use in medical application [55].

5. CONCLUSIONS

This study had a success with biologically synthesized nanoparticles. They can be obtained by low budget techniques and it can provide very good results. The Zinc oxide nanoparticles and silver nanoparticles have a predisposition to cause release of reactive oxygen species (ROS), and apoptosis within the cell. It makes zinc oxide nano particle a suitable candidate for anticancer

and antimicrobial activity [56]. It could be concluded that there was subsequently less damage to the liver with usage of Zinc oxide nanoparticles and silver nanoparticles in the presence of a toxic substance carbon tetrachloride that can easily cause a liver cirrhosis [57]. The weight of rats treated with Zinc oxide nano particle and silver nanoparticles increased over time, and the weight of rat that was administered with CCl4 had a perceivable difference due to the presence of liver cirrhosis inside the liver of this rat. Blood tests, i.e. Liver function test, Lactate dehydrogenase enzymes showed satisfactory data because there was some damage or a disease present inside the liver. Histopathological observation of an animal treated with Zinc oxide nanoparticles showed flawless results as compared to the animal that was administered with CCl4 only. Even though zinc oxide nanoparticles can help us as antimicrobial or anti-cancerous, but they have an issue of causing toxicity. Toxicity is by far the only issue that these nanoparticles have in common [58]. Zinc deposits itself in vital organs of our body. It can lead to further problems in long-term usage [59].

Author contributions: The study was conceptualized by Arham Dar and Hamza Noman, and was experimented, technically validated and the manuscript formatted. Abdullah Ghulam Nabi and Nayab Munawar helped in experimentation, collection and analysis of data. The literature review and background research was carried out by Hamza Noman. The methodology was designed and the figures made by Arham Dar. Hafiza Amna Ahmad helped in interpreting data, structure references and making the manuscript refined. Rahish conducted statistical test and wrote the results. Usman encouraged data visualization, proofreading and better overall manuscript clarity. The project was supervised, critically reviewed and approved by Mehreen Fatima.

Funding source: No external funding is received.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Adams PC, Bradley C, Frei JV. Hepatic zinc in hemochromatosis. Clin Invest Med. 1991;14:16–20.

Ademuyiwa O, Onitilo O, Dosumu O, Ayannuga O, Bakare A, Akinlatun W, Ogunyemi EO. Zinc in CCl4 toxicity. Biomed Environ Sci. 2002;15(3):187–95.

Akhtar MJ, Ahamed M, Kumar S, Khan MM, Ahmad J, Alrokayan SA. Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. Int J Nanomedicine. 2012;7:845–57. https://doi.org/10.2147/IJN.S29129

Alferah MAZ. Histological changes of male Wistar rats liver following the ingestion of zinc oxide nanoparticles with special emphasis on the histochemical alterations. J Histol Histopathol. 2018;5(1):4.

Anber NH, El-Ghannam MZ, El-Kheshen GA, Bialy MI. Evaluation of serum zinc level in Egyptian patients with hepatitis C-associated cirrhosis. J Pharm Biomed Sci. 2016;6(2):81–5.

Askgaard G, Grønbæk M, Kjær MS, Tjønneland A, Tolstrup JS. Alcohol drinking pattern and risk of alcoholic liver cirrhosis: a prospective cohort study. J Hepatol. 2015;62(5):1061–7.

Atukorala TM, Herath CA, Ramachandran S. Zinc and vitamin A status of alcoholics in a medical unit in Sri Lanka. Alcohol Alcohol. 1986;21:269–75.

Barry M, Keeling PW, Feely J. Tissue zinc status and drug elimination in patients with chronic liver disease. Clin Sci. 1990;78:547–9.

Barry MG, Macmathuna P, Younger K, Keeling PW, Feely J. Effect of zinc supplementation on oxidative drug metabolism in patients with hepatic cirrhosis. Br J Clin Pharmacol. 1991;31:488–91.

Baskol M, Ozbakir O, Coskun R, Baskol G, Saraymen R, Yucesoy M. Role of serum zinc and other factors on prevalence of muscle cramps in nonalcoholic cirrhotic patients. J Clin Gastroenterol. 2004;38:524–9.

Becheri A, Dürr M, Nostro PL, Baglioni P. Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. J Nanopart Res. 2008;10(4):679–89. https://doi.org/10.1007/s11051-007-9318-3

Bedi P, Kaur A. An overview on uses of zinc oxide nanoparticles. World J Pharm Pharm Sci. 2015;4(12):1177–95.

Moris D, Tsilimigras DI, Machairas N, Merath K, Cerullo M, Hasemaki N, Pawlik T. Laparoscopic synchronous resection of colorectal cancer and liver metastases: a systematic review. J Surg Oncol. 2019;119(1):30–9.

Mohsen H, El-Dahshan ESA, El-Horbaty ESM, Salem ABM. Classification using deep learning neural networks for brain tumors. Future Comput Inform J. 2018;3(1):68–71.

Mishra PK, Mishra H, Ekielski A, Talegaonkar S, Vaidya B. Zinc oxide nanoparticles: a promising nanomaterial for biomedical applications. Drug Discov Today. 2017;22(12):1825–33.

Mirzaei H, Darroudi M. Zinc oxide nanoparticles: biological synthesis and biomedical applications. Ceram Int. 2017;43(1):907–14.

Milman N, Hvid-Jacobsen K, Hegnhoj J, Sorensen SS. Zinc absorption in patients with compensated alcoholic cirrhosis. Scand J Gastroenterol. 1983;18:871–5.

Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Tainsky MA. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science. 1990;250(4985):1233–8.

Marchesini G, Bugianesi E, Ronchi M, Flamia R, Thomaseth K, Pacini G. Zinc supplementation improves glucose disposal in patients with cirrhosis. Metabolism. 1998;47:792–8.

Martinez-Hernandez A. The hepatic extracellular matrix. II. Electron immunohistochemical studies in rats with CCl4-induced cirrhosis. Lab Invest. 1985;53(2):166–86.

Pauzi N, Zain NM, Yusof NA. Microwave-assisted synthesis for environmentally ZnO nanoparticle synthesis. In: Proceedings of the 10th National Technical Seminar on Underwater System Technology 2018. Springer, Singapore; 2019. p. 541–6.

Parkes CM. Accuracy of predictions of survival in later stages of cancer. Br Med J. 1972;2(5804):29–31.

Mulder TP, Janssens AR, Verspaget HW, van Hattum J, Lamers CB. Metallothionein concentration in the liver of patients with Wilson's disease, primary biliary cirrhosis, and liver metastasis of colorectal cancer. J Hepatol. 1992;16:346–50.

Nakagawa H, Fujita M, Fujimoto A. Genome sequencing analysis of liver cancer for precision medicine. Semin Cancer Biol. 2019;55:120–7.

Ozbal E, Helvaci M, Kasirga E, Akdenizoglu F, Kizilgunesler A. Serum zinc as a factor predicting response to interferon alpha-2b therapy in children with chronic hepatitis B. Biol Trace Elem Res. 2002;90:31–8.

Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Kerem B. Nucleotide deficiency promotes genomic instability in early stages of cancer development. Cell. 2011;145(3):435–46. https://doi.org/10.1016/j.cell.2011.03.044

Farinati F, Cardin R, de Maria N, Lecis PE, Della Libera G, Burra P, Marafin C, Sturniolo GC, Naccarato R. Zinc, iron, and peroxidation in liver tissue: cumulative effects of alcohol consumption and virus-mediated damage. Biol Trace Elem Res. 1995;47:193–9.

Farnebo M, Bykov VJ, Wiman KG. The p53 tumor suppressor: a master regulator of diverse cellular processes and therapeutic target in cancer. Biochem Biophys Res Commun. 2010;396(1):85–9.

Goode HF, Kelleher J, Walker BE. Relation between zinc status and hepatic functional reserve in patients with liver disease. Gut. 1990;31:694–7.

Gronbaek K, Friis H, Feldman M, Sonne J, Andreasen PB. Lack of effect of zinc supplementation on antipyrine clearance in alcoholic liver disease. Eur J Clin Pharmacol. 1996;49:397–401.

Grungreiff K, Reinhold D. Liver cirrhosis and liver diabetes mellitus are linked by zinc deficiency. Med Hypotheses. 2005;64:316–7.

Grungreiff K, Abicht K, Kluge M, Presser HJ, Franke D, Kleine FD, Klauck S, Diete U. Clinical studies on zinc in chronic liver diseases. Z Gastroenterol. 1988;26:409–15.

Gur G, Bayraktar Y, Ozer D, Ozdogan M, Kayhan B. Determination of hepatic zinc content in chronic liver disease due to hepatitis B virus. Hepatogastroenterology. 1998;45:472–6.

Fukui H, Saito H, Ueno Y, Uto H, Obara K, Sakaida I, Tsubouchi H. Evidence-based clinical practice guidelines for liver cirrhosis. J Gastroenterol. 2016;51(7):629–50.

Hanley C, Layne J, Punnoose A, Reddy KM, Coombs I, Coombs A, Wingett D. Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles. Nanotechnology. 2008;19(29):295103. https://doi.org/10.1088/0957-4484/19/29/295103

El-Sayyad IH, Mohamed FI, Shalaby FM, Abou-El Magd RF, Rajiv GL, Fernando A, Raj HG, Ouhtit A. Histopathological effects of cisplatin, doxorubicin and 5-fluorouracil on the liver of male albino rats. Int Biol Sci. 2009;5(1):1–9.

He E, Qiu H, Huang X, Van Gestel CA, Qiu R. Different dynamic accumulation and toxicity of ZnO nanoparticles and ionic Zn in the soil sentinel organism Enchytraeus crypticus. Environ Pollut. 2019;245:510–8.

Hou J, Wu Y, Li X, Wei B, Li S, Wang X. Toxic effects of different types of zinc oxide nanoparticles on algae, plants, invertebrates, vertebrates and microorganisms. Chemosphere. 2018;193:852–60.

Hu CMJ, Fang RH, Wang KC, Luk BT, Thamphiwatana S, Dehaini D, Carpenter C. Nanoparticle biointerfacing by platelet membrane cloaking. Nature. 2015;526(7571):118–22.

Hussain Z, Khan JA, Anwar H, Andleeb N, Murtaza S, Ashar A, Arif I. Synthesis, characterization, and pharmacological evaluation of zinc oxide nanoparticles formulation. Toxicol Ind Health. 2018;34(11):753–63.

Hussain Z, Khan JA, Anwar H, Andleeb N, Murtaza S, Ashar A, Arif I. Synthesis, characterization, and pharmacological evaluation of zinc oxide nanoparticles formulation. Toxicol Ind Health. 2018;34(11):753–63.

Ijuin H. Evaluation of pancreatic exocrine function and zinc absorption in alcoholism. Kurume Med J. 1998;45:1–5.

Janssens AR, Bosman FT, Ruiter DJ, Van Den Hamer CJ. Immunohistochemical demonstration of cytoplasmic copper-associated protein in the liver in primary biliary cirrhosis: its identification as metallothionein. Liver. 1984;4:139–47.

Jiang J, Pi J, Cai J. The advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg Chem Appl. 2018;2018:1–10.

Kahn AM, Ozeran MD. Liver and serum zinc abnormalities in rats with cirrhosis. Gastroenterology. 1967;53:193–7.

Karayalcin S, Arcasoy A, Uzunalimoglu O. Zinc plasma levels after oral zinc tolerance test in nonalcoholic cirrhosis. Dig Dis Sci. 1988;33:1096–102.

Keeling PW, Jones RB, Hilton PJ, Thompson RP. Reduced leucocyte zinc in liver disease. Gut. 1980;21:561–4.

Keeling PW, Ruse W, Bull J, Hannigan B, Thompson RP. Direct measurement of hepatointestinal extraction of zinc in cirrhosis and hepatitis. Clin Sci. 1981;61:441–4.

Kiilerich S, Christiansen C. Distribution of serum zinc between albumin and alpha2-macroglobulin in patients with different zinc metabolic disorders. Clin Chim Acta. 1986;154:1–6.

Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide from synthesis to application: a review. Materials. 2014;7(4):2833–88.

Król A, Pomastowski P, Rafińska K, Railean-Plugaru V, Buszewski B. Zinc oxide nanoparticles: synthesis, antiseptic activity and toxicity mechanism. Adv Colloid Interface Sci. 2017;249:37–52.

Kugelmas M. Preliminary observation: oral zinc sulfate replacement is effective in treating muscle cramps in cirrhotic patients. J Am Coll Nutr. 2000;19:13–5.

Leveille CR, Arias IM. Pathophysiology and pharmacologic modulation of hepatic fibrosis. Vet Intern Med. 1993;7:73–84.

Lopes S, Ribeiro F, Wojnarowicz J, Łojkowski W, Jurkschat K, Crossley A, Loureiro S. Zinc oxide nanoparticles toxicity to Daphnia magna: size-dependent effects and dissolution. Environ Toxicol Chem. 2014;33(1):190–8.

Loza K, Epple M, Maskos M. Stability of nanoparticle dispersions and particle agglomeration. In: Biological Responses to Nanoscale Particles. Springer, Cham. 2019. p. 85–100.

Madden AM, Bradbury W, Morgan MY. Taste perception in cirrhosis: its relationship to circulating micronutrients and food preferences. Hepatology. 1997;26:40–8.

Maillard H, Celerier P, Maisonneuve C, Forest JL, Blanchi A, Pasquiou C. Necrolytic migrating erythema without glucagonoma. Ann Dermatol Venereol. 1995;122:786–8.

Marchesini G, Fabbri A, Bianchi G, Brizi M, Zoli M. Zinc supplementation and amino acid–nitrogen metabolism in patients with advanced cirrhosis. Hepatology. 1996;23:1084–9.

Marchesini G, Bugianesi E, Ronchi M, Flamia R, Thomaseth K, Pacini G. Zinc supplementation improves glucose disposal in patients with cirrhosis. Metabolism. 1998;47:792–8.