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The study of the impact of zinc oxide nanoparticles and carbon tetrachloride on the liver of *Rattus norvegicus*

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Abstract: Cancer is a prevalent problem in modern times and while there are available treatments, it continues to cause a significant number of deaths. Cancer is a complex anomaly of the body that is becoming increasingly difficult to control. In the field of nano medicine, nanoparticles are utilized as a drug transporter. Zinc oxide nanoparticles, in particular, offer flexible platforms for therapeutic and biomedical applications, and have the potential to act as an effective anti-cancerous drug. To investigate this potential, a study was conducted using *Rattus Norvegicus* as an animal model. The rats were administered carbon tetrachloride (CCl4) and zinc oxide (ZnO) nanoparticles for fourteen consecutive days. After euthanizing the rats, biomedical tests were performed to verify the therapeutic results of the nanoparticles. Analysis of histopathological slides and morphology revealed visible differences in the vital organ, with liver cirrhosis observed in the rats injected only with carbon tetrachloride compared to those injected with both carbon tetrachloride and zinc oxide nanoparticles. Overall, the study highlights the potential of zinc oxide nanoparticles as a promising anti-cancerous drug, with the need for continued research in the development of new and effective treatments for cancer.

Keywords: Nanoparticles, carbon tetrachloride, liver function, lactate dehydrogenase.

INTRODUCTION

Cancer is a serious disease that threatens lives, and curing it is one of the main tasks for scientists globally. Nanotechnology and nano medicines are one of the major fields that have exceptional abilities to control cancer. In a pilot-scale study, the primary concern was the common effect of zinc nanoparticles and carbon tetrachloride on the liver. Biologically synthesized nanoparticles were used in this study and the animal model was a typical lab rat, also known as *Rattus norvegicus* (Adams *et al*., 1991). The aim of the study was to determine the effect of nanoparticles on the rat in the presence of CCl_4, as the combination of toxic substances with the presence of nanoparticles could give us a better idea of the effectiveness of nanoparticles. If nanoparticles have any benefits, they will keep the liver healthy in the presence of CCl_4, otherwise, the liver will get damaged. Cancer is not a single disease but a group of diseases that can affect any part of the body. Cancer is a deformity inside the body caused by mutations in our DNA. Uncontrolled division of cells, resulting in immortality of cancerous cells, can give rise to a tumor (Ademuyiwa *et al*., 2002). The mutation can be caused by factors such as mutagens, insertion, deletion, or addition. A tumor can be either benign or malignant. Lymphomas, leukemia and myeloma are also other types of cancer.

The four stages of cancer are abnormal cells that may have the potential to convert into cancerous cells, cancerous cells present in a specific area, cancerous cells spreading towards nearby tissues and lymph nodes and metastasis cancer, where cancer spreads to other parts of the body.

Metastasis occurs when cancerous cells leave the tumor and flow into the bloodstream or lymphatic system. Genetic mutations are the basis of cancer, with genes controlling the main portion of cells. Acquired mutations are due to external harmful factors, such as radioactive radiation, UV light and tobacco, while germ line mutations can occur in sperm or eggs, affecting all cells in the body. The liver is a crucial organ in the body that plays a vital role in the metabolism process, filtering blood from the digested tract, metabolizing all medicines absorbed in the digestive tract, producing bile juice that aids digestion, and producing essential proteins that are responsible for proper body function (Akhtar *et al*., 2012). Liver cirrhosis is a severe disease that can cause death. It results from liver damage, which leads to the formation of scar tissue, causing abnormal functioning. Fig. 1 shows the difference between a normal liver and a liver affected by cirrhosis (Baskol *et al*., 2004).

Cancer remains a major problem, with available treatments not always effective, leading to a significant death toll. One of the main challenges is the resistance of tumors to drugs and the negative impact of chemotherapy

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and radiotherapy on healthy cells (Becheri *et al*., 2008). However, advances in nanotechnology and nanomedicine are offering new hope for the detection, diagnosis and treatment of cancer. Nanoparticles can be used to transport drugs and to attach directly to cancer cells, targeting only those that are affected while leaving healthy cells untouched. Zinc oxide nanoparticles, in particular, have been studied for their antimicrobial and anti-cancerous activities and have unique properties for drug delivery and other applications. However, the toxicity of nanoparticles, and the need to understand their properties and effects, remain important concerns (Cabre *et al*., 2008).

While many people are affected by cancer, and treatments have limitations, recent developments in nanotechnology are offering new possibilities. Nanoparticles can be used to carry drugs directly to cancer cells, avoiding damage to healthy cells, and targeting only those cells that are affected. This offers the potential for more effective treatments, without the side effects of chemotherapy and radiotherapy. Zinc oxide nanoparticles have been found to have unique properties and are being studied for their antimicrobial and anti-cancerous activities. These particles can be synthesized using eco-friendly methods, and are being explored for their potential in drug delivery and other applications (Callegari *et al*., 2019).

However, the toxicity of nanoparticles remains a concern, and it is important to understand their properties and effects in order to use them safely and effectively. Nanoparticles can be classified based on size and shape, using techniques such as x-ray diffraction (Capocaccia *et al*., 1991).

MATERIALS AND METHODS

In this experiment, the animal model used was the purebred albino laboratory rat, scientifically known as *Rattus norvegicus*. Three rats were selected for the study, each with nearly identical weights. The rats were then split into three distinct groups: A control group, a group treated with carbon tetrachloride to induce disease and a group treated with both zinc and carbon tetrachloride.

Formulation of nanoparticles

The carbon tetrachloride used in the study was a 99.5% anhydrous compound sourced from Sigma-Aldrich, which was imported from Steinheim am Albuch, Germany. The specific catalogue number for this compound was 32215. Sigma-Aldrich was also the brand of the product used to maintain a consistent pH level in the study, with a catalogue number of P5493 and sourced from Bavaria, Germany (Delaporte *et al*., 1997). For histopathology purposes, a 10% solution of formalin neutral buffer was used to suspend a portion of the liver after dissection. This solution had a catalogue number of HT501128 and was also sourced from Bavaria, Germany.

To dilute the carbon tetrachloride in a 1:1 volume ratio, olive oil was used in the study. The specific olive oil used was from Sigma-Aldrich and had a catalogue number of O1514, sourced from St Quentin Fallavier, France (Deterding *et al*., 2019).

Synthesis of nanoparticles

The study was conducted in the year of 2021-22 at university of Punjab. The zinc oxide nanoparticles were in a powdered form, and they were synthesized biologically. The zinc oxide nanoparticles were synthesized by sol gel method, this method involves the hydrolysis and condensation of metal alkoxide precursors in a solution to form nanoparticles. Zinc acetate or zinc nitrate can be used as a starting material, which is then mixed with a solvent and a stabilizer. The mixture is then heated to form a gel, which is dried and calcined to obtain zinc oxide nanoparticles. Reaction time varied for 2 and 3h with and without sodium acetate (0.01 M). In vitro studies using these particles had already been conducted and they had yielded successful results. The particles were accurately weighed on a measuring scale and then suspended in a 1.5ml microcentrifuge tube containing 1ml of PBS solution. Next, the particles were sonicated on a sonication machine for around two hours before undergoing vortexing for 20 minutes. For each day of dosage, the nanoparticles were placed in the sonication machine for one hour and vortexed for ten minutes. The required dosage for a rat weighing 150g was 0.75mg/150g/day, which was obtained by maintaining a stock of 5mg/kg/day. The final concentration for the rat was 50ul of ZnO nanoparticle. Carbon tetrachloride was taken in a 50ml falcon and mixed with pure 100% olive oil in a 1:1 concentration ratio. CCl4 is a toxic substance, and it must be handled with gloves to prevent skin irritation. The optimized concentration of CCl4 for each rat was 375ul/kg and for a 150g rat, the dosage was 56.25ul. The CCl4 group rat weighed 164g on day zero and received a dose of 61.5ul of CCl4, while the Zinc Oxide + CCl4 rat weighed 142g on day zero and received a dose of 5tabl5ul of CCl4. One rat was administered CCl4 and ZnO nanoparticles for 14 days according to its weight, while another rat was given CCl4 alone for 14 days. A control group of rats received the same diet and water as the other rats, but no injections were given to them to monitor the condition of a normal rat. After 14 days, all rats were dissected, and their liver organ and blood were collected for histopathology slides and blood tests, respectively. The blood was taken directly from the heart by pumping it into a 10cc syringe and the liver was removed and fixed in formalin for liver histopathology.

The aspartate transaminase (AST) test utilized the ASAT (GOT) FS* reagent kit, which is an optimized UV test that follows the guidelines of the International Federation of Clinical and Laboratory Medicine. The alanine aminotransferases (ALT) test was conducted using a kinetic test that adheres to the guidelines of the International Federation of Clinical and Laboratory Medicine.

The total bilirubin test was performed using 2, 4 dichloroaniline (DCA). To obtain the necessary reagents, please refer to table 1, which provides the catalogue number and the quantity of reagents needed.

Table 1: Catalogue number and reagents quantity 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

The alkaline phosphatase test was a photometric test optimized using the standard method outlined by the German Society of Clinical Chemistry (DGKC). Table 1 contains the catalogue number and amount of reagents required for ordering purposes.

Ethical approval
The studies were conducted on murine models and authors declared that there are no ethical issues in this paper.

STATISTICAL ANALYSIS

The analysis of study was conducted on SPSS2.0 software by using ANOVA.

RESULTS

Rat weight
The weight of an individual's body is subject to internal changes. This study focuses on liver cirrhosis induced by CCl_4 as the main factor, which resulted in changes to the body weight of rats within a 14-day period. This is evidenced here in this study.

Table 2: Shows the weight of rats in grams from day zero to day fourteen.

Days	0	2	4	6	8	10	12	14
Control	135	146	156	167	177	187	197	198
CCl_4+Zn	143	163	163	172	194	207	209	208
CCl_4	170	183	183	166	169	175	172	178

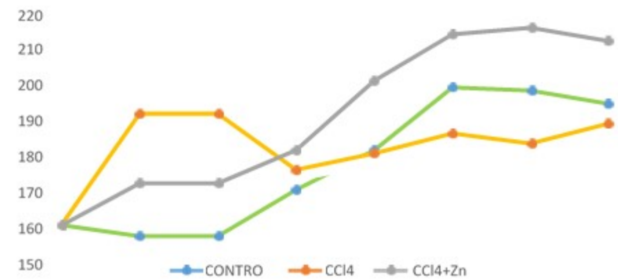

Fig. 1: The weight of the rats was recorded and presented in the weight data, with the weight of the rat measured in grams and plotted on the y-axis. As compared to the combination or control rats, a significant decrease in the weight of the rats was observed.

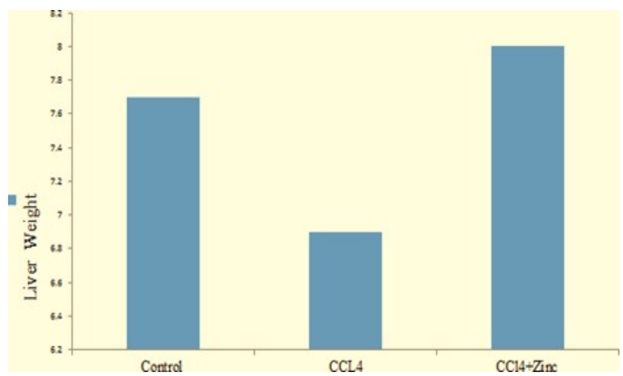

Fig. 2: This chart displays the liver weight of rats, with the groups of rats indicated on the x-axis ($p \leq 0.05$) and their respective weights on the y-axis.

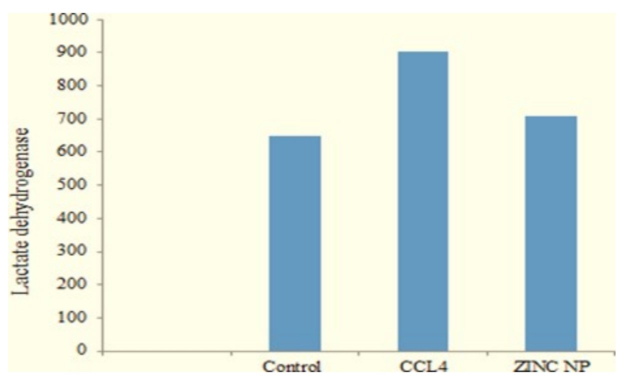

Fig. 3: The LDH values of the rats are presented on the graph, with the rat groups listed on the x-axis and their respective LDH values on the y-axis ($p \leq 0.05$). Rats administered with CCl_4* had significantly higher LDH values compared to those in the control group or those administered with CCl4 and Zinc oxide nanoparticles.

Liver weights
The administration of CCl4 and the combination group also had an impact on the liver weights of the rats. The liver samples were collected for chemical analysis, with the control group represented by the blue line, the CCl_4 diseased group by the red line and the group receiving a

combination of CCl4 and Zinc nanoparticles by the grey line. A significant decrease in the liver weight was observed in the rats induced with CCl4 as compared to those administered with CCl4 and Zinc oxide nanoparticles. Additionally, the rats in the control group had a higher weight than those induced with CCl_4.

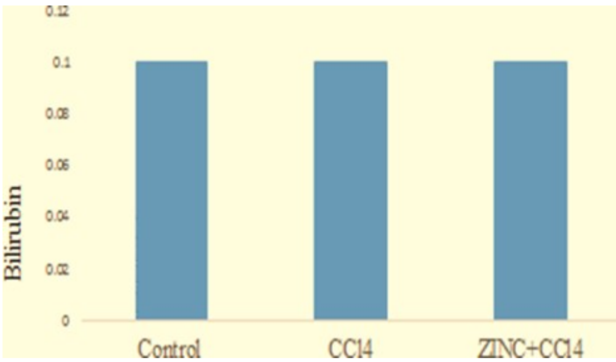

Fig. 4: The bilirubin total inside the liver. On the x-axis rats are taken, and on they-axis values of bilirubin test are taken. There is no difference inany value.

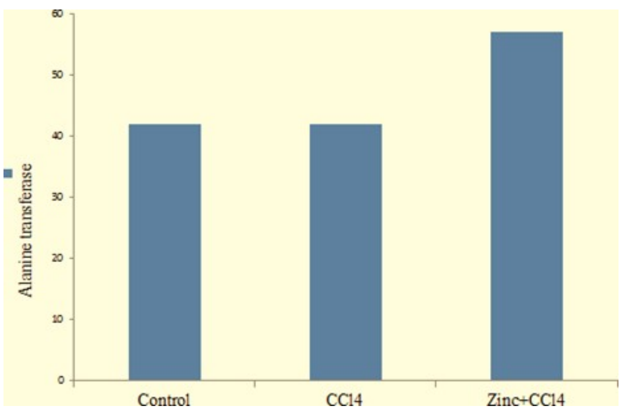

Fig. 5: The graph displays the ALT values of rats, with ALT representing the alanine transferase enzyme, which is an indicator of liver damage ($p \leq 0.05$). The y-axis shows the ALT test values, while the x-axis represents a corresponding measurement, Zinc+CCl_4*

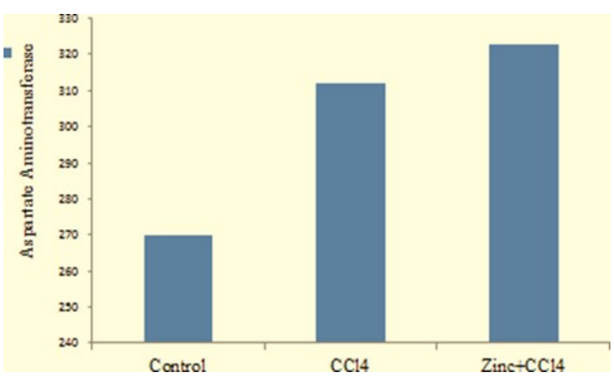

Fig.6:Y-axispresents AST testvaluesandx-axispresentsrats groups($p \leq 0.05$) and significant difference in Zinc+CCl_4* and CCl_4*.

Comparison of lactate dehydrogenase eenzyme

In comparison to the combination group, the administration of CCl_4 resulted in a significant increase in LDH levels in rats, indicating liver damage and organ injury.

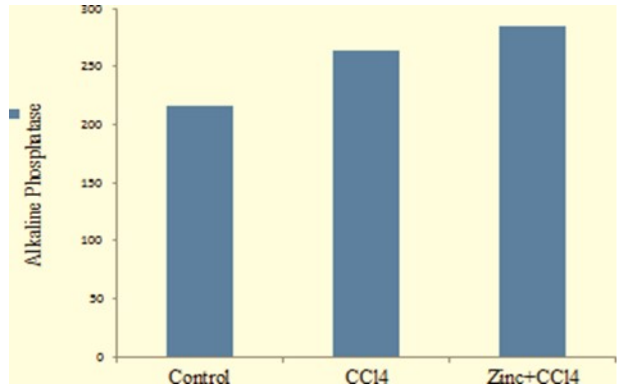

Fig. 7: y-axis shows the value of ALP, x-axis present groups of rat ($p \leq 0.05$).CCl_4+ZINC*

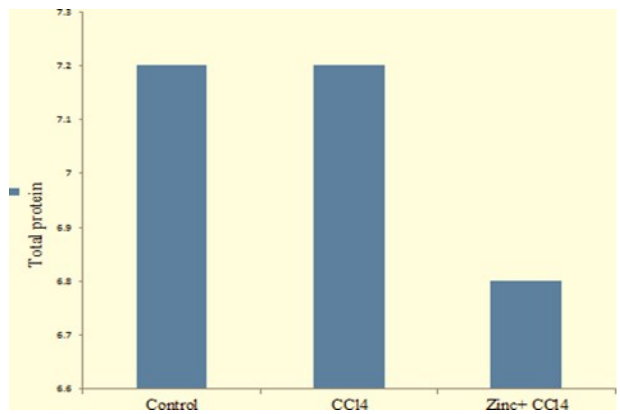

Fig. 8: y-axis includes values of total protein count and x-axis includes rats group ($p \leq 0.05$) and significant difference in Zinc+CCl_4*.

Amount of bilirubintotal

Bilirubin normal values in the red blood cells. Otherwise high values indication to produce liver cirrhosis and much other disease in liver.

Alaninetransferase enzyme

Liver injury introduced by CCl_4 and ALT graph shows the normal values of liver in combination group as compare to the CCl_4 group.

Comparison of aspartateaminotransferase

AST stands for Aspartate Aminotransferase enzyme. It releases when there isdamage to the liver. This enzyme value are high in this graph as compare to the CCl_4 group but here is no big difference that indicate liver damage with zinc nanoparticles.

Comparison of alkalinephosphatase

It is known as ALP test. High levels of ALP shows 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.

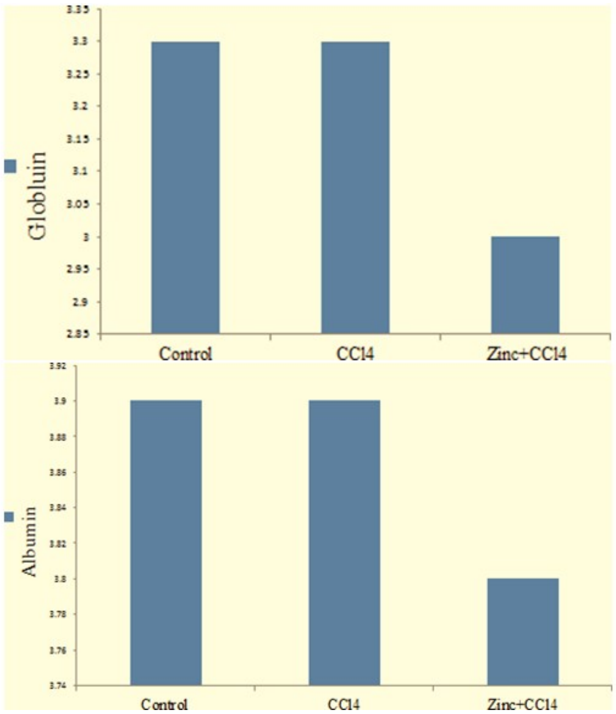

Fig. 9: On x-axis rats are present; on y-axis values of Albuminand Globulinare present ($p\leq0.05$) significance difference in Zinc+CCl_4*.

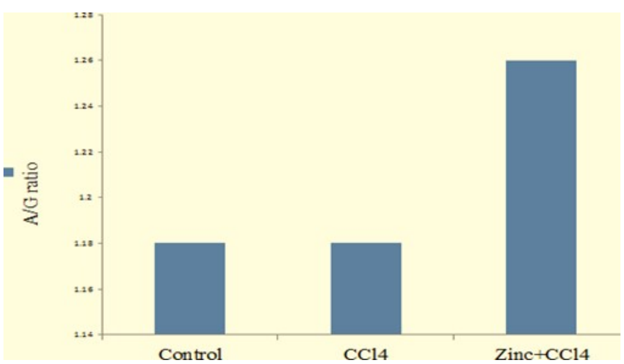

Fig. 10: This figure illustrates the Albumin and Globulin ratio, where a high A/G ratio indicates liver damage ($p\leq 0.05$) significance difference in Zinc+CCl_4*. The y-axis represents the values of the A/G ratio, while the x-axis lists the rats in the study. Rat number one represents the control group, rat number two was administered with CCl_4 and Zinc oxide nanoparticles, and rat number three was administered with CCl4, showing a relatively higher A/G value compared to the other rats.

Amount of 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.

Comparison of albuminand globulin

A total protein count is a combination of both albumin and globulin. If there are low number of total protein count than there is a clear indication of a liver damage

Ratio of A/G

The Albumin and Globulin ratio, where a high A/G ratio indicates liver damage. However, based on the chemical analysis, it appears that there is relatively less damage to the liver overall.

DISCUSSION

Liver cirrhosis is an irreversible disorder that occurs due to consistent scarring of liver tissues and continuous regeneration, resulting in a loss of structural integrity of nodules (Elshama *et al*., 2018). Major factors that contribute to liver cirrhosis include viral infections, alcohol, and fatty liver. Liver cirrhosis has a high risk of developing into cancer, which can be fatal. The use of nanotechnology in medical fields has been increasing due to the development of nanomaterials and their various applications (Farinati *et al*., 1995). Viral diseases like hepatitis advanced stages always welcome to the cirrhosis and become cancer on advance stages. Moreover, liver develop this dangerous diseases also with in fatty liver (Farnebo *et al*., 2010). The nanotechnology is spreading the entire world because of nanomaterial development and so many applications. With other treatments of cancer they kill healthy cell but with this nanotechnology they can't kill healthy cell that is why nanotechnology consider being successful from last five years the zinc demand rapidly rise in medical field (Galon *et al*., 2012). This study is related to the nanotechnology that shows effective and successful results in liver cirrhosis of the Rats. Zinc nanoparticles were used to kill liver damage called liver cirrhosis (Garrett *et al*., 2019). A study has shown that zinc nanoparticles effectively treat liver cirrhosis in rats without harming healthy cells, making it a promising anti-cirrhosis drug for the future (Fukui *et al*., 2018). Chronic liver diseases, including viral infections like hepatitis and other factors such as alcohol and obesity, can lead to liver fibrosis, which is a reversible process treatable with medication (Hassan *et al*., 2009). If liver fibrosis persists for an extended period, it can result in irreversible liver cirrhosis, which can cause severe damage to the liver. Zinc has been found to have a protective mechanism that protects the liver from liver fibrosis and can help restore and support liver pathways for those suffering from liver cirrhosis. Zinc supplements have also been found to reduce muscle cramps and aid in glucose metabolism. Zinc deficiency can cause various diseases, including growth retardation, neurological issues and delayed wound healing, while an excess of zinc can cause toxicity. Studies have found that zinc deficiency is widespread in cirrhosis patients with higher disease severity (Smaoui *et al*., 2023).

CONCLUSION

In this study, researchers found that biologically synthesized nanoparticles are a promising solution for low-budget and effective results. Zinc oxide nanoparticles in particular have been shown to induce a release of reactive oxygen species and apoptosis within cells, making them a suitable candidate for anticancer and antimicrobial activity. Additionally, in the presence of a toxic substance like carbon tetrachloride that can cause liver cirrhosis, the use of zinc oxide nanoparticles resulted in less damage to the liver and positive outcomes in terms of weight gain and blood tests. However, it is important to note that while zinc oxide nanoparticles have potential benefits, they also have issues with toxicity and the deposition of zinc in vital organs over long-term usage. Despite the potential benefits of using zinc oxide nanoparticles as antimicrobial and anticancer agents, toxicity is a common issue associated with these nanoparticles. Specifically, the deposition of zinc in vital organs can lead to further health problems in the long term (Zhang *et al*., 2023). While the study showed positive outcomes in terms of reducing liver damage, weight gain, and blood test results in rats, it is important to consider the potential risks and side effects associated with the use of these nanoparticles. Therefore, further research is necessary to fully understand the implications of using zinc oxide nanoparticles as a therapeutic agent (Abdelghany *et al*., 2023).

Future perspectives

Zinc oxide nanoparticles offer promising potential for targeted delivery in medical applications. By combining with aptamers, these nanoparticles can be directed towards specific cells, such as cancer cells, while leaving healthy cells unharmed. This strategy effectively addresses the issue of toxicity associated with nanoparticles. Additionally, zinc oxide nanoparticles can be modified to include probes that selectively detect cells with high levels of survivin protein, which is typically abundant in cancer cells. This development has the potential to revolutionize the field of medicine, allowing for more effective and precise treatments (Usman *et al*., 2023).

Furthermore, the use of zinc oxide nanoparticles in early stage liver cancer research shows that this model may be a valuable tool for studying the occurrence and development of liver cancer. With the ability to target specific cells and detect specific proteins, zinc oxide nanoparticles offer a promising approach for early detection and treatment of cancer. As a result, these nanoparticles have the potential to significantly impact the future of medicine (Wong*et al* 2018: Azam*et al*., 2023).

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