Journal of Population Therapeutics & Clinical Pharmacology

DOI: 10.53555/jptcp.v30i17.3773

THE STUDY OF THE IMPACT OF GOLD NANOPARTICLES AND CARBON TETRACHLORIDE ON THE LIVER OF RATTUS NORVEGICUS

Abdullah Qadir[1], Mirza Shaharyar[2*], Madiha Afzal[3], Iqra Khalid[4], Muhammed Abid[5], Sabahat Saeed[6], Noman Waheed[6], Talha Ashfaq[7], Aqsa Sadiq[8], Shamim Akhtar[9]

[1]Quaid-e- Azam Medical College Bahawalpur, Pakistan
[2]Lahore Medical and Dental College, Pakistan
[3]Department of Physics, University of Engineering and Technology, Lahore, Pakistan
[4]Department of Zoology wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan
[5]Department of Entomology The Islamia University Bahawalpur
[6]Department of Zoology, Hazara University Mansehra, Pakistan
[6]Department of Chemistry, University of Agriculture Faisalabad, Pakistan
[7]Department of Chemical Sciences, University of Padova Italy
[8]Department Entomological research institute. Ayub Agricultural Research institute. Faisalabad
***corresponding Author**

Abstract

Gold nanoparticles have been widely studied in nanotechnology for their anti-cancer properties and other potential benefits. In this study, gold nanoparticles were evaluated for their therapeutic effects on the liver injury caused by CCl4 in *Rattus Norvegicus*. Male rats were induced with liver injury through CCl4 and treated with gold nanoparticles daily for 14 days. To determine the therapeutic effects, biochemical analysis was performed, and after 14 days, the rats were euthanized. The results showed that the rats injected with CCl4 had experienced weight loss and fur color changes, whereas the rats injected with CCl4 and gold nanoparticles had increased weight and normal feeding habits. This suggests that gold nanoparticles have anti-inflammatory effects and can alleviate liver cirrhosis. This study provides evidence for the potential therapeutic applications of gold nanoparticles in liver injury treatment.

Keys Words: Gold Nanoparticles, Carbon Tetrachloride, *Rattus Norvegicus,* Liver
Corresponding Author: Madiha Afzal (Madihaafzal228@gmail.com)

Introduction

Cancer is a devastating disease that has caused numerous deaths worldwide. According to Anand et al. (2008), the death toll in 2018 alone was approximately 9.6 million. GLOBOCAN 2018 estimates an even higher number of cases, with around 18.1 million people affected. Shockingly, even in low HDI settings, 1 in 10 women and 1 in 8 men are likely to develop cancer in their lifetime. Cancer cells tend to grow and divide uncontrollably, requiring oxygen and nutrients from the body. They can spread from one part of the body to another through the lymph nodes, which are clusters of immune cells present throughout the body. Cancer can arise from various genetic mutations, including those affecting cell cycle genes, DNA damage repair

genes, cell apoptosis genes, and genes responsible for growth regulation. Cancer is classified into three main types: sarcomas, carcinomas, and leukemias, with over 200 recognized subtypes to date (Arvizo et al., 2010). Nanotechnology is a rapidly advancing field with significant applications in various industries. Among the different nanoparticles manufactured through nanotechnology, gold nanoparticles hold a unique advantage due to their size, shape, and ease of transmission. They can be utilized in the pharmaceutical industry and have demonstrated potential in managing microbial infections and cancer treatment. Although there are not enough studies performed on the size and shape of gold nanoparticles, their active use in cancer treatment is evident. Their small size enables them to easily penetrate tumor sites and target cancer cells, while also binding to drugs and proteins. Additionally, gold nanoparticles are biocompatible, but their preparations can be toxic in both in vivo and in vitro systems. They produce strong contrasts during imaging due to their high atomic number, which allows for effective x-ray absorption. Gold nanoparticles are a crucial element in various fields of biotechnology and pharmacology. Pure colloidal gold has been known to have a beneficial impact on weak organs, particularly the digestive system, by reducing cancer mass and improving blood circulation. Additionally, gold nanoparticles have proven effective in treating arthritis, tuberculosis, and in the healing of burns, scrapes, and open sores. In 1983, the World Health Organization (WHO) recognized gold as a food additive. Recent medical research has found that gold nanoparticles have unique properties that enable them to identify cancer cells, making them an ideal vector for diagnostic and chemotherapeutic substances. Additionally, gold nanoparticles can detect biological molecules such as enzymes, proteins, DNA, antibodies, and antigens. An experiment was conducted to examine the effect of biosynthesized gold nanoparticles on liver cirrhosis in rats induced with CCl4. Nanoparticles possess various properties that make them useful in a wide range of biomedical fields. For instance, they can act as biosensors or chemical sensors, emitting magnetic fields that are utilized in magnetic resonance imaging (MRI) and other magnetic-related applications. Nanoparticles also exhibit catalytic properties, which are influenced by their size and shape, with smaller nanoparticles generally having better catalytic activities (Bartlomiejczyk et al., 2013). Additionally, they display good electrical conduction, which again depends on their size and shape. These diverse properties of nanoparticles hold great potential for humankind, although much remains unknown regarding their biological properties, with limited data available on their biological applications. Nevertheless, there are known anti-microbial properties of nanoparticles, which could benefit pharmaceutical industries, and the recent discovery of their anti-cancer effects promises a vital role for future anti-cancer medicines. Metal nanoparticles possess a large surface area that allows for the conjugation of multiple medicines, enabling targeted delivery to specific sites within the body. Nanoparticles can also be referred to as Theragnostic, combining therapeutic and diagnostic capabilities.

The field of cancer nanotechnology has seen incredible advancements in understanding the biomedical properties of nanoparticles, particularly with the emergence of gold nanoparticles. Gold nanoparticles come in various structures, including nano rods, shells, cages, and stars, which are beneficial for different biomedical applications. Nano rods, cages, and shells are useful for photothermal therapy due to their strong light absorption in the nearinfrared therapeutic window, while star-shaped gold nanoparticles enhance Raman spectroscopy imaging. The synthesis and optical properties of gold nanoparticles are essential in enabling the binding of ligands or conjugation of medicines for diagnosis or therapy (Bartneck et al.,

2014). Gold nanoparticles can also be targeted by attaching targeting substances to their surfaces, with fully controlled size splitting of their various nano structures. While previous research highlighted the potential of gold nanoparticles in cancer mono-therapies, there is now increasing interest in their use in combination therapies, including chemotherapy, radiotherapy, hyperthermia, photothermal therapy, dual-modality cancer therapy, thermo-radio therapy association, and triple-modality cancer therapy (Bataller et al., 2003).

Chronic liver diseases (CLD) refer to diseases where the liver is chronically affected and may lead to liver fibrosis, cirrhosis, or cancer. While the liver has the capacity to regenerate and repair damaged tissues, several factors can contribute to liver damage, including diseases, viruses, medicines, or excessive alcohol consumption. Chronic infections like hepatitis, jaundice, hepatitis B, and hepatitis C can severely damage the liver, as can fatty liver due to excessive alcohol consumption. Medicines like metronidazole or acetaminophen taken for prolonged periods can also cause CLD. There are no available medicines in the market for liver anti-fibrosis. Many therapies have failed to cure liver diseases effectively. However, therapies involving nanoparticles have shown better effects than conventional therapies. Nanotechnology has enabled the conjugation of nanoparticles with biological compounds or drugs that can target specific parts of the body. The use of nanoparticles for drug delivery systems has shown great potential in the past few years for curing various liver diseases (Bednarski et al., 2015).

The liver is one of the largest organs in the human body, weighing around 1.5kg in a healthy adult. The liver produces bile juice stored in the gallbladder, which helps digest food. The liver is the only organ with the capacity to regenerate damaged tissues, even up to 70% damage. However, continuous injury may lead to liver fibrosis, where excess extracellular matrix accumulates in the liver tissue, causing severe structural and functional changes in the liver. CLD is a major concern worldwide, and a damaged liver can lead to critical conditions like liver cirrhosis (Carnovale et al., 2015).

Methodology

The animal model used in the study was a laboratory albino rat with the biological name of *Rattus Norvegicus*. Three rats were selected based on their similar weight and pure breed, and were divided into three groups: Normal, carbon tetrachloride, and gold and carbon tetrachloride.

The gold nanoparticles used in the experiment were prepared at university of Punjab Lahore, Pakistan for duration of 1 year. These nanoparticles were mycogenized. To prepare the stock solution, the gold nanoparticles were mixed with 1ml of PBS. The mixture was vortexed for 20 minutes using a vortex device to ensure that the liquid was thoroughly mixed. After vortexing, the liquid was subjected to sonication for 1 hour to break up any aggregates of small-sized colloidal gold particles. The carbon tetrachloride used in the study was anhydrous with a purity of 99.5%. It was purchased from Sigma-Aldrich in Steinheim am Albuch, Germany, with a catalogue number of 32215.Phosphate-buffered saline (PBS) was used in the experiment to maintain a continuous pH level. The PBS brand used was SigmaAldrich, with a catalogue number of P5493, from Bavaria, Germany. Formalin neutral buffer 10% solution was used to suspend the liver after dissection. The formalin was purchased from Bavaria, Germany, with a catalogue number of HT501128.

Olive oil and carbon tetrachloride were mixed in equal parts [1:1] and placed in a 1.5mL microcentrifuge tube. The mixture was then vortexed for 20 minutes before preparing gold

nanoparticles in the same manner. Following mixing and vertexing, the mixture was sonicated for one hour (Castro et al., 1975).

Experimental design

One rat received CCl4 and ZnO nanoparticles for fourteen days based on its weight, while another rat received only CCl4 for the same duration, also based on its weight. The purpose of the latter was to serve as a comparison for the rat's condition without nanoparticles. A control group was also established, which received the same diet and water as the other rats, but did not receive any injections. This group served as a baseline for the rats' normal condition (Doria et al., 2016).

Animal model	**Treatment given**
One rat	Control /No treatment
one rat experimental	CCL4 dose given 1:1
one rat experimental	CCl4 with Gold nanoparticles 1:1

Table 1: Experimental design on rat model

Sample collection

The rats were sacrificed for experimental analysis after 14 days, and blood was directly collected from their hearts. To test for LFT and LDH, 5 to 10 ml of blood was drawn using 10cc syringes. Once blood was collected from the rats, their liver was removed from the body and immersed in formalin to undergo histological analysis. This was done to accurately diagnose cancer tumors in the CCl4 group and study the major effects of gold nanoparticles on the tumors.

ASPARTATE TRANSAMINASE (AST)

This AST kit was used for test that optimized UV test according to the international Federation of clinical and laboratory medicine (Dreaden et al., 2012).

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

Table 2: This table shows catalogue number, size of kit and order information

ALANINE AMINOTRANSFERASES (ALT)

Test was perfumed according to the International Federation of clinical and laboratory medicine.

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

Table 3: The Table shows catalogue number and reagents for order information

TOTAL BILIRUBIN (BIL)

In Total bilirubin test were performed by using 2,4 dichloroaniline (DCA).

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

Table 4: The table shows catalogue number and quantity for order information.

ALKALINE PHOSPHATASE (ALP)

The test was photometric optimized standard method.

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: This table shows catalogue number and reagent quantity for order information

Results

WEIGHT OF RATS

Cirrhosis in rats can be indicated by their body weight, as it tends to differ noticeably between those suffering from the condition and those that are healthy. A significant shift in weight may signify liver damage. The analysis of study was conducted on SPSS2.0 software by using ANOVA.

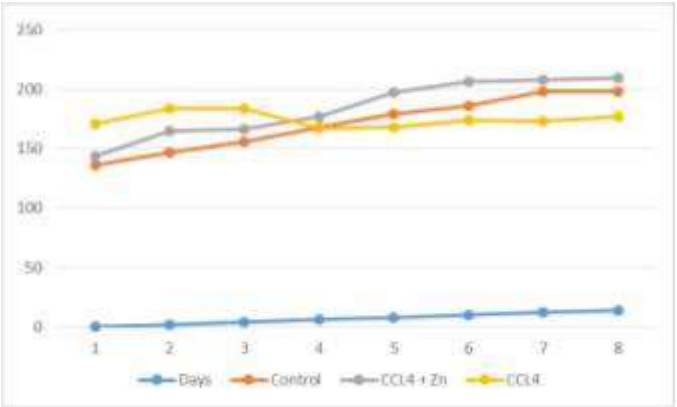

Figure 1: Experiment design on rat weight during 14 days ($p \leq 0.05$). Weight of rat is present on y-axis and days are present on the x-axis.

LIVERS WEIGHT

The changes in liver weight caused by CCl4 were used to assess the condition of the rats in groups A and B. Group A was subjected to both control and CCl4, while group B was given a combination of CCl4 and gold nanoparticles.

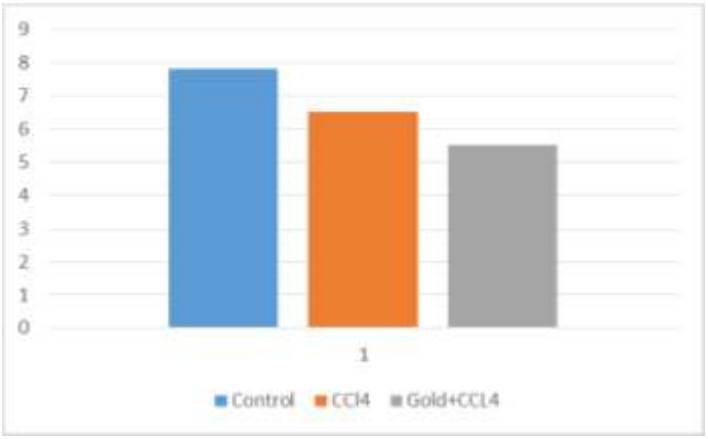

Figure 2: **Comparison** indicates the liver weight of CCl4, CCl4 and gold nanoparticles and the control group ($p \leq 0.05$)

LACTATE DEHYDROGENASE (LDH)

The LDH test demonstrated greater damage in the rat liver of the CCl4 group, which serves as a marker of oxidative stress. An increase in LDH levels suggests a higher likelihood of cirrhosis compared to the combination group, where no toxicity was induced in the rat liver. Graphical results indicate that gold nanoparticles are the most effective in preventing liver cirrhosis, as there was no indication of cirrhosis.

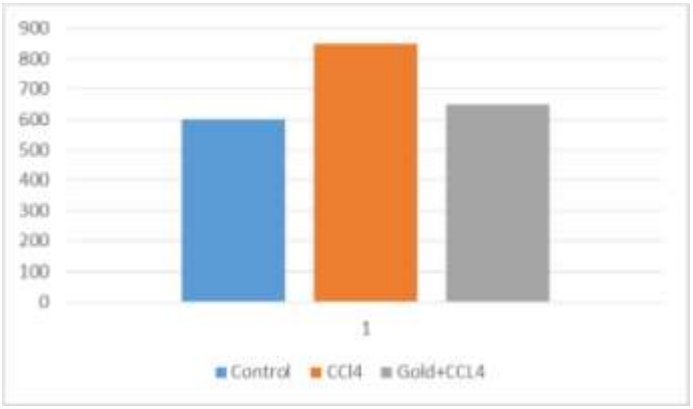

Figure 3: Comparison of LDH of control and experimental group on the y-axis there is LDH values and x-axis shows Rat groups ($p \leq 0.05$).

BILIRUBIN TOTAL

Essentially, bilirubin is a measure of the regular degradation of red blood cells. While ALP levels are typically normal in cirrhosis, bilirubin levels can become elevated in numerous liver diseases as it is produced in the liver. Total bilirubin levels tend to remain stable until a more advanced stage of liver disease is reached.

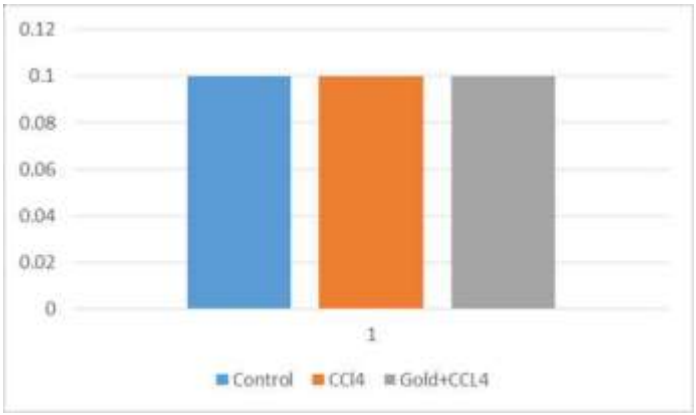

Figure 4: Comparison of total Bilirubin in the liver are on the x-axis and the y-axis showing groups of the rats ($p \leq 0.05$).

ALANINE TRANSFERASE ENZYMES

The Alanine aminotransferase (ALT) test is a blood test used to detect liver injury. In this graph, the combination group exhibits low levels of ALT, indicating that the liver is functioning normally and suggesting that gold nanoparticles, in combination with CCl4, are effective in improving and suppressing liver damage. High levels of ALT typically indicate liver damage.

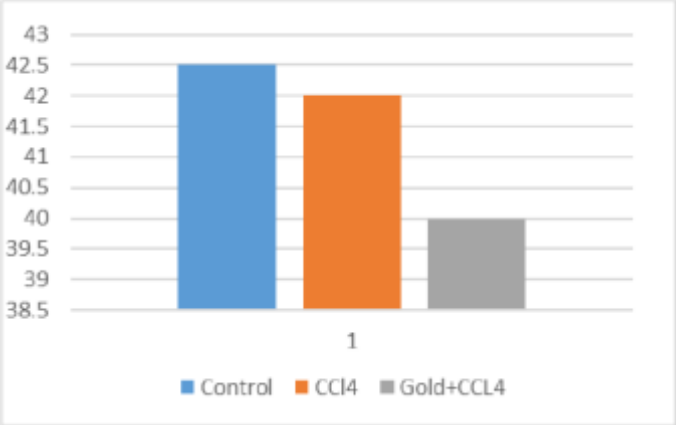

Figure 5: Comparison of ALT in control and experimental groups ($p \leq 0.05$)

Aspartate Amino transferase Enzymes

AST is predominantly present in the liver and muscles throughout the body. Elevated levels of this serum in the bloodstream indicate liver damage. The results here suggest that there may be some minor liver damage in the combination group, but it is not entirely conclusive as there is no significant difference between the diseased group and the combination group.

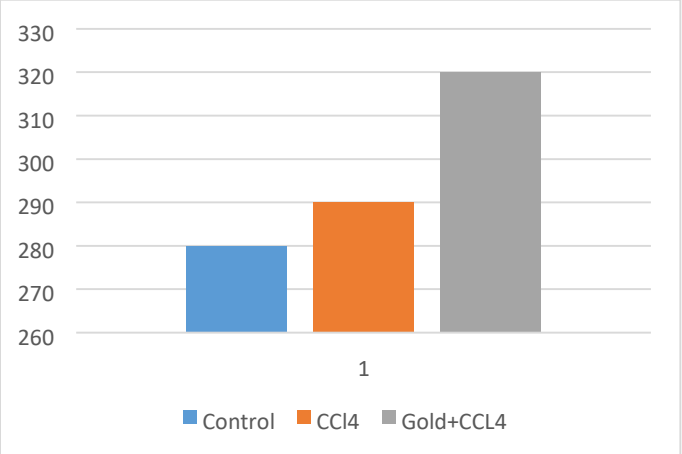

Figure 6: Comparison of AST of liver which means Aspartate Amino transferase Enzymes ($p \leq 0.05$).

ALKALINE PHOSPHATE

The Alkaline phosphatase level in this test is normal, which suggests that the alkaline level in the bloodstream is within the normal range and that there are no signs of cirrhosis in the rat liver in the combination group. Compared to the CCl4 group, the liver in the combination group does not appear to have any significant problems.

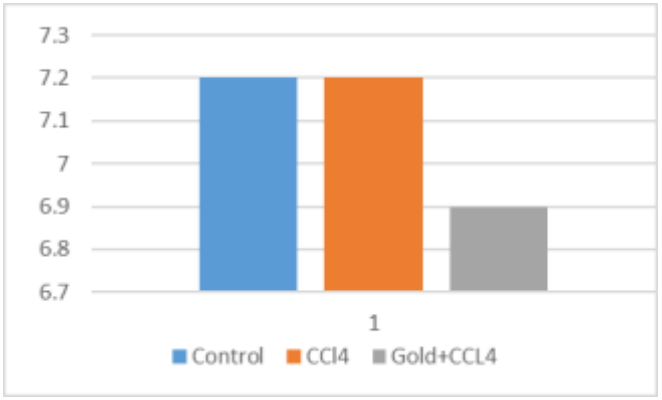

Figure 8: Comparison of the high-level APL damage to the liver between control and experimental ($p \leq 0.05$).

TOTAL PROTEIN

The combination group shows normal levels of total protein in the blood as compared to the CCl4 group, which can aid in the diagnosis of liver diseases.

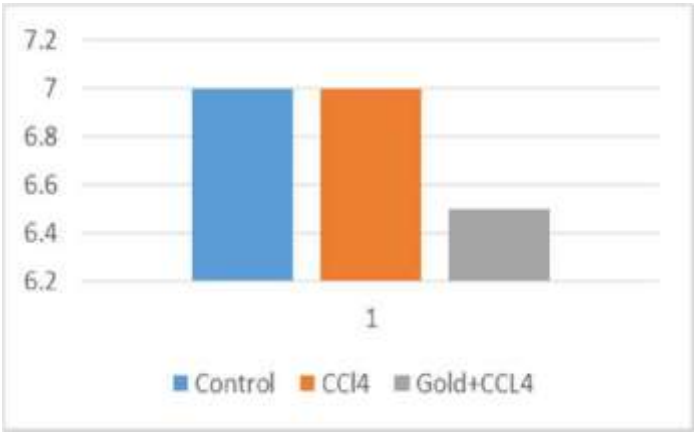

Figure 9: Comparison of total protein values are on the y-axis and the group of rats are on xaxis ($p \leq 0.05$). Total protein shows same value in the first two group control and CCl4 and the less protein value in the gold nanoparticles and CCl4.

ALBUMIN AND GLOBLUIN

The combination of these two graphs shows normal values of the combination group which indicates the normal value of protein.

Ratio of A/G

This test was also look at the ratio of albumin to globulin in blood. This is known as the "A/G ratio." It shows comparison of albumin and globulin proteins.

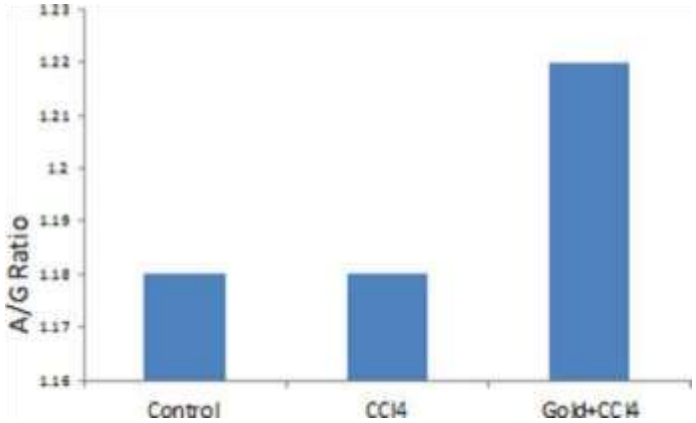

Figure 10: Comparison of A/G ratio on y-Axis the Ratio of the A/G value and the x-axis show the groups of the control and experimental ($p \leq 0.05$).

Discussion

Nanotechnology is considered to be a breakthrough technology in curing deadly diseases, including cancer. Among the many nanoparticles, gold nanoparticles have a significant impact on the field of molecular biology, due to their specific toxicity inside the cancer cells. Unlike the traditional treatments of cancer, such as chemotherapy and radiotherapy, which not only affect cancer cells but also healthy cells, gold nanoparticles can target only the cancer cells and leave the healthy cells unharmed (Elci et al., 2016). Gold nanoparticles can be used to treat cancers that are located near vital organs where surgery may not be an option. Nanoparticles, in general, have various applications in biotechnology, pharmacology, and other industries. The size of nanoparticles is crucial, as it affects their effectiveness. Nanoparticles that are too large can damage the cellular membrane and blood vessel linings, whereas those that are too small may not reach inside the desired cell. Therefore, the sizing of nanoparticles is a critical factor in determining their efficacy (Elle et al., 2013). Nanoparticles are modified by different molecular engineering processes and can be combined with medicine to form a single structure, known as ENPs, for nano medicine. ENPs have a high surface area that makes them useful for the diagnosis of various diseases, such as tumor detection, gene-delivery therapies, and treatments. Metallic nanoparticles have a specific area on their surface that can provide conjugation between a nanoparticle and medicine. This makes it easy for medicine to be delivered to the desired point. Gold nanoparticles are a recent addition to the biological application of nanoparticles, but they have a significant impact on bio sciences (Galon et al., 2012). They can be used for drug delivery, diagnostics, cancer and microbial related disease treatments, and disease prevention. Gold nanoparticles have different abilities, depending on their size (Ghosh et al., 2008). They have the ability to emit optical properties and have been used in physics for plasmonic concepts, such as near-field and far-field enhancement between the electron and nucleus of noble metals. The shape and structural change of gold nanoparticles can help to tune their plasmonic behavior and provide a visible spectrum of infrared light, reducing the cost of adding additional probe to the gold nanoparticle to give fluorescent light (Ghosh et al., 2014). Gold has been used in Ayurveda for a long time, and it has

also been used in some dishes as an edible (Zhang et al., 2023). While gold in compound form can be harmful to the body, 100% pure gold is less harmful. Colloidal gold or gold in nanoparticle form does not cause toxicity at any level because it is pure gold (Abdelghany et al., 2023). Although many studies on gold nanoparticles focus on therapies and drug carriers, modifying these particles could help to utilize them for various other purposes. In conclusion, gold nanoparticles are a promising entity for nano medicines and have the potential to revolutionize the treatment of cancer and other deadly diseases (Usman et al., 2023).

Conclusion

According to the findings of this study, the use of gold nanoparticles in the liver has been successful. The tumors disappeared in the group C on the liver surface, which was very clear and shiny, similar to the control group rats. This study suggests that nanotechnology can be utilized to reduce cancer cells in the liver. Despite being an expensive technology, the gold nanoparticles used in this study were synthesized biologically, which is novel. Moreover, the study utilized a low-budget biosynthesized gold nanoparticle as an anti-cancer agent in the nanotechnology field, which produced remarkable results in repairing liver damage caused by CCl4. The use of gold nanoparticles as an anti-cancer drug has shown fewer side effects on organs, making it a powerful alternative to traditional methods. Gold nanoparticles are also known to be less toxic in the nanotechnology field.

In conclusion, the results of this study demonstrate the potential of gold nanoparticles as an effective anti-cancer agent in liver cirrhosis. By optimizing the use of gold nanoparticles, we can potentially develop new and innovative treatments for liver cancer that have fewer side effects and a greater impact on the overall health of patients.

Ethical approval

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

References

1. Anand P, AB Kunnumakara, C Sundaram, KB Harikumar, ST Tharakan, OS Lai and BB Aggarwal (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharma Res*. **25(9):2097-2116.**
2. Arvizo R, R Bhattacharya and P Mukherjee (2010). Gold nanoparticles: opportunities and challenges in nanomedicine. *Expert opin drug deliv*. **7(6):753- 763.**
3. Bartlomiejczyk T, A Lankoff, M Kruszewski and I Szumiel (2013). Silver nanoparticles allies or adversaries. *Ann Agri EnvironMed*. **20(1) :223-225 .**
4. Bartneck M, KT Warzecha and F Tacke (2014). Therapeutic targeting of liver inflammation and fibrosis by nanomedicine. Hepatobiliary surg nutri. **3(6): 364.**
5. Bataller R, KE North and DA Brenner (2003). Genetic polymorphisms and the progression of liver fibrosis: *a crit appra*. **37:493-503.**

6. Bednarski M, M Dudek, J Knutelska, L Nowiński, J Sapa, M Zygmunt and M Tęsiorowski (2015). The influence of the route of administration of gold nanoparticles on their tissue distribution and basic biochemical parameters: in vivo studies. *Pharm Rep*. **67(3):405-409.**

7. Carnovale C, G Bryant, R Shukla amd Bansal V (2016). Size, shape and surface chemistry of nano-gold dictate its cellular interactions, uptake and toxicity. *Progress Mats Sci*. **22(83):152-190.**

8. Carnovale C, G Bryant, R Shukla and V Bansal (2016). Size, shape and surface chemistry of nano-gold dictate its cellular interactions, uptake and toxicity. *Progress Mats Sci*. **23(83):152-190.**

9. Castro JA, EC De Ferreyra, CR De Castro, OM De Fenos, H Sasame and JR Gillette (1974). Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism—further studies on their mechanism of action. *Bioch Pharma*. **23(2):295302**.

10. Doria G, J Conde, B Veigas, L Giestas, C Almeida, M Assunção and PV Baptista (2012). Noble metal nanoparticles for biosensing applications. Sensors. **12(2):1657-1687**.

11. Dreaden EC, A M Alkilany, X Huang, CJ Murphy and MA El-Sayed (2012). The golden age: gold nanoparticles for biomedicine *Chem soc rev*. **41(7):2740-2779.**

12. Elci SG, Y Jiang, B Yan, ST Kim, K Saha, DF Moyano and RW Vachet (2016).

Surface charge controls the suborgan biodistributions of gold nanoparticles. *ACS nano*.

10(5):5536-5542.

13. Elle RE, S Gaillet, J Vide, C Romain, C Lauret, N Rugani and JM Rouanet (2013). Dietary exposure to silver nanoparticles in Sprague–Dawley rats: effects on oxidative stress and inflammation. *Food chem toxi*. **12(60):297-301.**

14. Galon J, F Pagès, FM Marincola, HK Angell, M Thurin, A Lugli and F Tatangelo (2012). Cancer classification using the Immunoscore: a worldwide task force. *J. trans medi*. **10(1):205.**

15. Ghosh P, G Han, M De, CK Kim and VM Rotello (2008). Gold nanoparticles in delivery applications. *Adv drug rev*. **60(11):1307-1315.**

16. Ghosh S, J Sengupta, P Datta and A Gomes (2014). Hematopoietic and antioxidant activities of gold nanoparticles synthesized by aqueous extract of fenugreek (Trigonella foenum-graecum) seed. *Adv Sci Med*. **6(5):546-552.**

17. Zhang, W., Sani, M. A., Zhang, Z., McClements, D. J., & Jafari, S. M. (2023). High performance biopolymeric packaging films containing zinc oxide nanoparticles for fresh food preservation: A review. *Inter J Bio Macro*.**36 (5): 123-188**.

18. Abdelghany, T. M., Al-Rajhi, A. M., Yahya, R., Bakri, M. M., Al Abboud, M. A., Yahya, R and Salem, S. S. (2023). Phytofabrication of zinc oxide nanoparticles with advanced

characterization and its antioxidant, anticancer, and antimicrobial activity against pathogenic microorganisms. *Bio Conversion.* **13(1), 417-430.**

19. Usman, M., Zia-ur-Rehman, M., Rizwan, M., Abbas, T., Ayub, M. A., Naeem, A., and Ali, S. (2023). Effect of soil texture and zinc oxide nanoparticles on growth and accumulation of cadmium by wheat: a life cycle study. *Environ Research,* **(216) 51: 114-120.**