Pak. J. Agri. Sci., Vol. 61(3), 923-929;2024
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/24.299
http://www.pakjas.com.pk

Anti-Proliferative Effect of Biosynthesized Zinc-Oxide Nanoparticles Using *Albizia Lebbeck* Leaves Extract Against Arsenic-Induced Liver Cancer in Rats

Khursheed Haroon[1], Faiza Hassan[1*], Muhammad Rehan Sajid[1], Tanzila Sahar[2], Misbah Ijaz[3], Muhammad Tahir Mohyud-din[3], Asad Manzoor[3], Mahrukh Babar[1], Aqsa Rashid[1] and Kashif Iqbal[1]

[1]Institute of Physiology and Pharmacology, FVS, University of Agriculture Faisalabad; [2]Department of Biochemistry Govt. College Women University, Faisalabad; [3]Department of Clinical Medicine and Surgery, FVS, University of Agriculture, Faisalabad, Pakistan
*Corresponding author's e-mail: faiza.hassan@uaf.edu.pk

The most common type of cancer is hepatocellular carcinoma (HCC). The current study aimed to induce hepatocellular injury in the Wistar rat model through the administration of arsenic at a dose rate of 5mg/kg. In the context of treating induced liver toxicity, biocompatible nanocomposites were proposed as a drug delivery system. The proposed intervention involved administering a nanomaterial consisting of zinc oxide and *Albizia lebbeck* intraperitoneal at a dose rate of 10 mg/kg for 8 weeks. For this research, 24 Wistar rats were divided into four distinct groups. Except for the normal control group, rats from each group were administered intraperitoneal injections of arsenic oxide every other day for 56 days, at a dosage of 5 milligrams per kilogram of body weight, to induce hepatocellular carcinoma. The HCC that has been developed went under evaluation through various methods, including histopathology, complete blood count, health markers, and ultrasonography. The decrease in HCC dimensions was ascertained through the use of ultrasonography. The statistical analysis of the acquired data was involved the use of ANOVA and the least significant difference at a significance level of 5%.
Keywords: Hepatocellular carcinoma, Arsenic, Nanocomposites, Ultrasonography, Histopathology.

INTRODUCTION

The liver is a vital organ that helps the body with immunity, digestion, detoxification, metabolism and vitamin storage among other functions. It accounts for roughly 2% of an adult's body weight. Hepatocellular carcinoma (HCC) in humans has been associated with various risk factors, including cirrhosis, alcoholic and non-alcoholic fatty liver as well as persistent infections (Motta *et al*., 2023).Exposure to certain chemicals, specifically arsenic may lead to some serious health concerns from acute adverse side effects to chronic diseases. Arsenic-induced carcinoma is becoming a major health issue nowadays. In addition to cancer, arsenic may also contribute to the production of some serious ailments including skin lesions, hypertension, ischemia, certain endemic peripheral vascular illnesses, diabetes, severe arteriosclerosis and neuropathies (Martinez *et al*., 2011).

Numerous herbal remedies are mentioned in Ayurveda and other complementary medicines, but their acceptance and application in improving the general health of the public are still in their early stages. Common names for *Albizia (A.) Lebbeck* include Indian Siris and East Indian Walnut. *A. Lebbeck*includes cytotoxic and anticancer properties. The *Albizia* genus contains triterpenoid saponins. Complex triterpenoid saponins, such as adianthifoliosides, grandibracteosides, gummiferaosides and albizosides have been isolated from the Albizia genus. Tumor cell growth is restricted by these potential anticancer natural triterpenoid saponins.Glycoside components called saponins are frequently referred to as "natural detergent" because of their frothy consistency. The anti-carcinogenic, immune-modulating and cell-proliferative properties of saponins, along with their potential to decrease cholesterol have all been demonstrated (Noté *et al*., 2015).

In the present study, liver cancer was induced with arsenic in a rat model and the biocompatible nanocomposite of zinc oxide of *A. lebbeck* (ZnO-Al-NPs) was used as a drug and it was compared with the efficiency of the standard anticancer drug (Anuk *et al*., 2022; Liang-liang *et al*., 2023). No study is available on the anti-proliferative effect of ZnO-Al-NPs against arsenic-induced liver damage in rats. Therefore, This study's goals were to prepare and characterize ZnO-Al-NPs,

Haroon, K., F. Hassan, M. R. Sajid, T. Sahar, M. Ijaz, M. T. Mohyud-din, A. Manzoor, M. Babar, A. Rashid and K. Iqbal. 2024. Anti-Proliferative Effect of Biosynthesized Zinc-Oxide Nanoparticles Using Albizia Lebbeck Leaves Extract Against Arsenic-Induced Liver Cancer in Rats. Pakistan Journal of Agricultural Sciences 61:923-929.
[Received 12 Sep 2024; Accepted 26 Sep 2024; Published 28 Sep 2024]

to evaluate the antiproliferative effects and track the benefits as neuroprotectants in albino rats (Mansour *et al*., 2023; Malik *et al*., 2022).

MATERIALS AND METHODS

***Chemical*:** ZnO-Al-NPs were prepared in the postgraduate Pharmacology lab in UAF, Faisalabad. Normal Saline was purchased from the care pharmacy in Faisalabad. Arsenic Trioxide and Fluorouracil Standard Drugs were purchased from SIGMA-ALDRICH.

***Preparation of A. lebbeck plant extract*:** *A. lebbeck* leaves were collected rinsed with running tap water after arriving from the University of Agriculture Faisalabad, kept in the shade for air drying and then crushed by a grinder to obtain powder 56 ml of ethanol and 80 g of dry crushed leaves were combined and the mixture was allowed to sit for 48 hours prior to two hours of heating and agitation at 45°C. The plant extract was then twice filtered using Whatman filter paper (Desai and Taranath, 2022).

***Synthesis ofZnO-Al-NPs*:** The prepared extract was directly used in the synthesis experiments. 0.1 M of zinc nitrate and 1ml Plant was dissolved in 500 ml of deionized water and heated at 60°C for 5 minutes on a hot plate stirrer.Then added 0.2M NaOH solution dropwise with burette which lead to milky and cloudy appearance stirred for 2h at 60°C. The suspension was spinned for 10 minutes at 10,000 rpm., which lead to white pellet formation. These pellets were first washed by distill water, purified by centrifugation and then with ethanol. A hot air furnace was used to dry the precipitate at 60°C until dried NPs were obtained. For 2 hours, the prepared NPs were calcined at 450°C.

***Characterization ofZnO-Al-NPs*:** Characterization of nanoparticles was executed at Central Hitch Laboratory, Government College University, Faisalabad. Crystalline properties were determined and purity of nano ZnO, a Jeol JDX-3532 diffractometer (Japan) was used. Electron Microscopy has been done by Quanta 250 FEG (USA). The composition of constructed materials was calculated by Fourier Transform Infrared Spectroscopy (FTIR) Bruker IFS 125HR Japan (Umair *et al*., 2022; Azam *et al*., 2022). Particle size was defined by Dynamic Light Scattering using a zeta particle sizer (Nano ZS - Melvern, USA).

Experimental animal: Twenty-four (24) male Wistar rats were chosen for the experimental trial with weights ranging from 200 ± 20 g (age 8-12 weeks). All the animals were kept at room temperature (25–27 °C), humidity (45–70%) and light–dark cycle (12 hours) throughout the study in animal house of UAF, Pakistan. Adjustment time was given for 15 days. Fresh feed was furnished to rats twice a day and water ad libitum was given. Guidelines for the care of laboratory animals were adhered to during the entire experiment according to directorate research studies.

EXPERIMENTAL DESIGN

This study comprised of two control and two treatment groups, each having six rats.Group I animals were kept as a normal control given with normal routine diet. Group 2 animals were kept as a positive control and rats were injected with arsenic intraperitoneally at a dose rate of 5mg per kg body weight (Mansour *et al*., 2023). Group 3 was the standard drug group in which rats were injected with Fluorouracil to treat HCC at a dose rate of 25mg per kg body weight. Group 4 was a treatment group in which rats were injected with ZnO-Al-NPs nanocomposite for treatment of HCC at a dose rate of 10mg per kg body weight.

***Blood sampling*:** For the total blood count, a sample of blood was drawn from the Juglar vein and placed in an EDTA tube. To prevent clotting, the sample was gently shook to combine the blood with EDTA. The samples were kept in a box intended for cold storage. In order to obtain serum, blood was drawn from an additional animal location, placed in gel clot activator tubes, and centrifuged for ten minutes at 3000 rpm (Centrifugal Machine; China). Serum was collected using Eppendorf tubes and stored in the freezer at 4°C for the evaluation of biochemical parameters.

***Biochemical considerations*:** The Alanine transaminase (U/L), Aspartate transaminase (U/L), Creatinine, Urea, Uric acid and complete blood count (CBC) were all calculated using the clear serum obtained after centrifugation (Akpinar *et al*., 2023). The automatic Biochemistry analyzer(make model) was used to measure all the components. ALT and AST levels are considered biomarkers for the indication of hepatic cancer.

***Histopathology*:** After both the induction and the treatment, a histopathological investigation was carried out. Following sample washing in regular saline (0.9% NaCl), obtained specimens were preserved in 10% formalin (Shaker*et al*., 2022). Use paraffin wax to impregnate the sample. A light microscope was used to examine the samples and (hematoxylin and eosin) stains used. H & E stain provided a comprehensive study of samples (Yoo *et al*., 2022).

STATISTICAL ANALYSIS

A one-way ANOVA test was used to evaluate the collected data and the least significant (LSD) test was used to estimate the data means with a 5% probability using Minitab version 20 of a statistical computer program.

RESULTS

***Characterization of ZnO-Al-NPs*:** Characterization of green synthesized ZnO-Al-NPs was done by using XRD, FTIR, Zeta size, Zeta potential and SEM. In XRD 2 higher values were revealed i.e 100nm at 33°, 002nm at 35°, 101nm at 37°, 102nm at 47°, 110nm at 57°, 103nm at 64°, 200nm at 67°, 112nm at 69°, 201nm at 70°, 004nm at 73°, 204nm at 77° indicating it to be in a hexagonal shape. In FTIR peaks were recorded that were 3332cm^{-1}, 1735cm^{-1}, 1402cm^{-1},1413cm^{-1},

647 cm^{-1}. *this study was compared with (*Zhou*et al*., 2023) as shown in Fig.1.

Table 1. Identification of various functional groups through FTIR. **Each functional group has its own discrete vibrational energy which can be used to identify a molecule through the combination of all of the functional groups.**

FTIR recorded value	Functional group
3332 cm^{-1}	O-H
1735 cm^{-1}	C=O
1402 cm^{-1}	C-C-H
1413 cm^{-1}	Isopropyl group
647 cm^{-1}	Zn-O link

The zeta size of ZnO-Al-NPs was recorded 66nm in range between 47-100 d.nm. In zeta potential 2 higher peaks were recorded -25Mv and +25Mv indicating that nanocomposite are stable in nature. SEM showed that ZnO-Al-NPs were non-cryltalline and round in shape with diameter of 65.12nm.

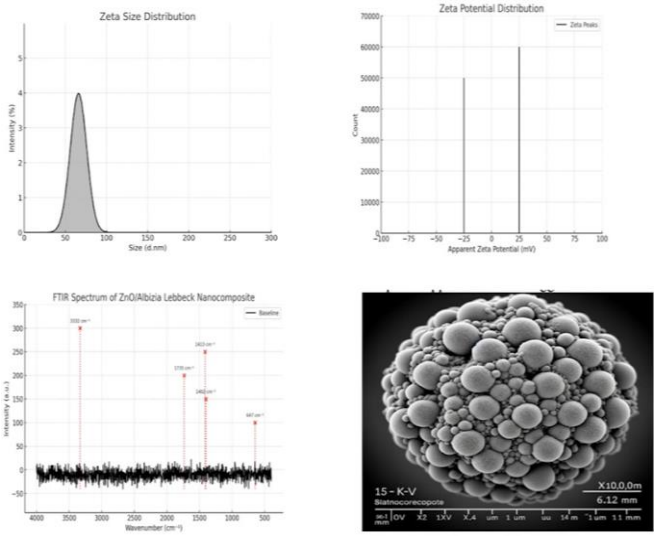

Figure 1. Characterization of ZnO-Al-NPs showing Zeta size, Zeta potential, FTIR and SEM. They have unique physical and chemical properties due to their small size, high surface area-to-volume ratio, and ability to absorb and scatter light in the visible and near-infrared range.

***Effects on kidney function parameters*:** The serum creatinine (mg/dl), urea (mg/dl) and uric acid (mg/dL) mean ± SD values are displayed in Fig. 2. The information reveals that there are substantial differences ($P < 0.0001$) in the mean ± SD values of serum creatinine, urea, and BUN among the treatment groups. There was a time and dose dependent increasing trend in the creatinine, urea and uric acid concentrations in the arsenic-treated group. In the arsenic-treated group, high levels of serum creatinine, urea and uric acid were noted at weeks three and four.In treatment group with ZnO-Al-NPs; creatinine, urea and uric acid levels decreased as compared to the arsenic-treated group as shown in Fig. 2.

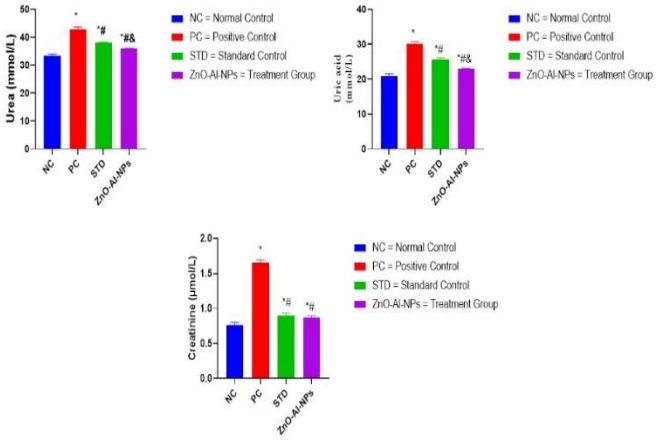

Figure 2. Effect of ZnO-Al-NPs on kidney function parameters by comparing all four groups on the basis of urea, uric acid and creatinine. Where * = significance of Normal control with other groups, # = significance of Positive control with treatment groups and & = significance ofStandard control with Treatment group.

***Effects on LFTs parameters*:** Data shows that the normal control group, which presumably did not receive any treatment, showed a negligible increase in Aspartate Aminotransferase (AST) levels from the 8th to the 12th week, indicating stable enzyme activity. The positive control group, which might have been subjected to a condition expected to increase AST levels without any therapeutic intervention, also showed a very slight increase.Reflecting a consistent elevation in AST activity. Both ZnO- Al-NPs and the standard drug treated groups exhibited a significant reduction in AST levels after treatment. The ZnO-Al-NPs treated group showed a more pronounced decrease. The serum ALT levels in the arsenic-induced liver cancer rat group were substantially higher than in the control group. When compared with arsenic-induced liver cancer group, ZnO-Al-NPs produced a substantial reduction in ALT (U/L) levels as shown in Fig.3.

***Effect of ZnO- Al-NPs on RBCs, hemoglobin, and WBCs*:** The data shows that the ZnO-Al-NPs (trial drug) had a stabilizing effect on RBC and hemoglobin levels, maintaining them close to those of the normal control, which suggests it does not adversely affect erythropoiesis or hemoglobin synthesis. Additionally, it notably lowered WBC counts compared to the positive control, which could indicate a potential anti-inflammatory or immunomodulatory effect. In contrast, the standard drug Fluorouracil resulted in an increase in hemoglobin but also a moderate increase in WBCs, which

could suggest a different mechanism of action or side effect profile in comparison to the ZnO-Al-NPs as shown in Fig. 4.

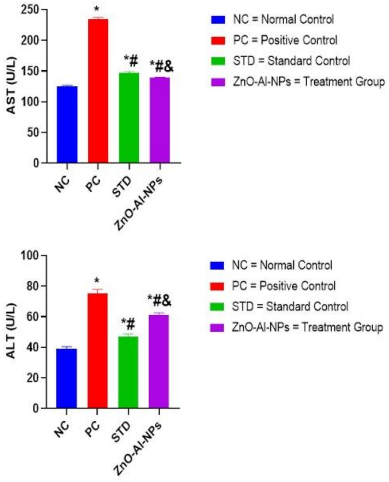

Figure 3. Effect of ZnO-Al-NPs on Liver functions parameters by comparing all four groups on the basis of AST and ALT. Where * = significance of Normal control with other groups, # = significance of Positive control with treatment groups and & = significance of Standard control with Treatment group

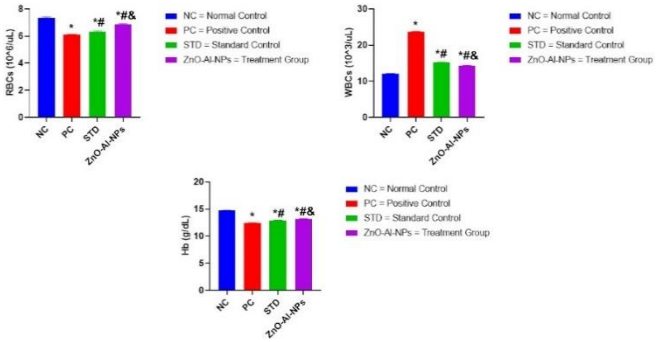

Figure 4. Effect of ZnO-Al-NPs on CBC by mainly focusing on values of RBCs, WBCs, and Hb all four groups.Where * = significance of Normal control with other groups, # = significance of Positive control with treatment groups and & = significance of Standard control with Treatment group

***Ultrasonic evaluation*:** An F-value of 13.86 and a p-value of 0.00 for the ultrasonic examination conducted before to treatment imply that the factors under test have a very significant impact on the outcome variable. With such a low p-value, we can conclude with high confidence that the variation between the different levels of the factors is not due to chance, and these factors indeed have a substantial effect on the ultrasonic measures being assessed.

Table 2. Showing ultrasonic evaluation results by dividing groups into pre-treatment and post-treatment groups of parenchymal echotexture, presence of nodules and surface margins mean SD.

Treatment	Parenchymal Echotexture		Presence of Nodules		Surface margins Mean SD	
	Pre	Post	Pre	Post	Pre	Post
Nagative control	0	0	0	0	0	0.00
	0	0	0	0	0	
	0	0	0	0	0	
	0	0	0	0	0	
	0	0	0	0	0	
	0	0	0	0	0	
Positive control	2	2	1	1	1	1.278
	2	2	1	1	1	
	2	2	0	0	1	
	2	2	1	1	1	
	2	2	1	1	1	
	2	2	1	1	1	
ZnO-*Albizia Lebbeck* (Al)-NPs nanocomposite	2	2	1	1	1	1.167
	1	1	0	0	1	
	2	2	0	0	1	
	2	2	1	1	1	
	2	2	1	1	1	
	2	2	1	1	1	
Fluorouracil	2	1	1	0	0	0.111
	1	0	1	0	0	
	2	0	0	0	0	
	2	0	1	0	0	
	2	0	1	0	0	
	2	1	1	0	0	

Pre = Pre-treatment; Post = Post-treatment

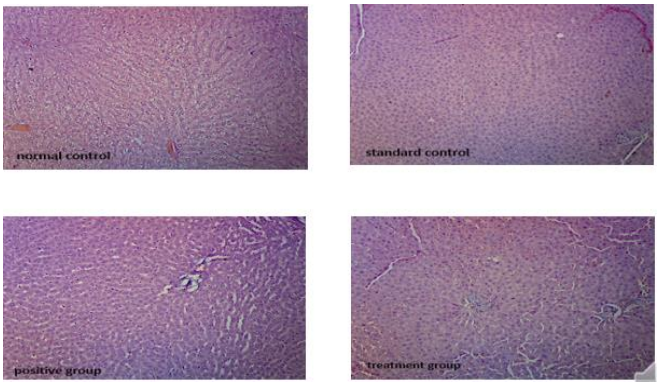

Figure 5. Effect of ZnO-Al-NPs nanocomposite on histopathology of liver in normal control group, standard group, positive control group and treatment group. Histopathological alterations such as fibrosis, fatty change, and multilobular nodules were improved in ZnO-Al-NPs group.

***Histopathological examination*:** HCC induced by arsenic administration and protection by ZnO- Al-NPs was examined by histopathological examination of liver of representative rats of each group. The central vein and surrounding

hepatocytes of control rats have typical histological changes. Histological examination of liver tissues in arsenic treated rats revealed focal regions in the hepatic parenchyma with fibrosis, fatty change and multilobular nodules. The hepatic parenchyma in the ZnO-Al-NPs group showed regular hepatic cords, normal nuclei of hepatocytes with standard sinusoidal spaces and mild vacuolar degeneration as shown in Fig. 5.

DISCUSSION

This research evaluated the therapeutic effect of ZnO-Al-NPs nanocomposite against arsenic-induced hepatocellular carcinoma in albino rats. Characterization of green synthesized ZnO- Al-NPs nanocomposite were done by using XRD, FTIR, Zeta size, Zeta potential and SEM. In XRD 2 higher values were revealed, indicating it to be in hexagonal shape (Naser *et al*., 2024; Samy *et al*., 2022; Shahid *et al*., 2023).The FTIR analysis revealed the following peaks: 3332 cm-1, 1735 cm-1, 1402 cm-1, 1413 cm-1, and 647 cm-1 (Naser *et al*., 2024; Anjum*et al*., 2023).The zeta size of ZnO-Al-NPs was recorded 66nm in range between 47-100 d.nm. Although most nanoparticles are between 1 and 100 nm in size, certain research shows that they can reach up to 1000 nm in size (Altammar, 2023). The rate of medication release and absorption can be influenced by the size of the nanoparticle. The surface area will rise if the size is too tiny, which will ultimately increase the drug's release. The stirring speed may also affect the particle size (Jayachandran*et al*., 2021).

One technique to ascertain the surface charge of the nanoparticles in solution (colloids) is zeta potential analysis. The colloidal stability is determined by the zeta potential's magnitude. Monodispersity is caused by a high negative or positive zeta potential greater than 30 mV. Agglomeration results from lower values, those less than 5 mV. Van Der Waal inter-particle attractions will eventually induce dispersions with lower zeta potential values to agglomerate. Zeta potential is important for understanding the surface condition of nanoparticles and predicting their long-term stability (Kamble *et al*., 2022). In zeta potential 2 higher peaks were recorded -25Mv and +25Mv indicating that nanocomposites were stable.

To describe the molecular makeup of ZnO-Al-NPs, FTIR studies of ZnO-Al-NPs were performed. FTIR spectrum has been shown in Fig. 2. The readings were nearly identical, indicating that the medication and the polymers were well suited to each other. The presence of a hydroxyl group was indicated by a strong peak (OH). However, at 2 values C=O group was present. At second peak isopropyl group was recorded and at other ZnO linkage was present. The peaks identified in our investigation were similar to those found in (Srinivasan*et al*., 2024). SEM showed that the image of ZnO-Al-NPs was non-crystalline and sphere in shape with diameter of 65.12nm. (Frase*et al*.,2007).A noteworthy rise ($P < 0.0001$) was observed in the study's serum creatinine and uric acid levels. urea and serum creatinine are the typical metrics used to assess renal function (Sindi *et al*., 2023). While urea is created in the liver from amino acids, creatinine is a waste product of creatinine phosphate metabolism that is made in the muscles (Astawibawa *et al*., 2022; Akbudak *et al*., 2023). Increased urea and creatinine levels indicate possible renal injury and a deterioration in kidney function. Increased levels of creatinine and urea result from kidney cell breakdown, which worsens the filtration system (Haryono *et al*., 2018). In arsenic-treated animals, there was an increase in blood creatinine and uric acid levels.. This study was in corespondant to (Zheng *et al*., 2022).

The data indicates a substantial difference ($P < 0.0001$) in the mean ± SD values of serum creatinine, urea and uric acid among the therapy groups. The concentrations of creatinine, ureaand uric acid in the group treated with arsenic showed a rising trend, measuring 1.65±0.04, 38.10±0.15, and 25.77±0.30, respectively. There were elevated serum creatinine levels in the therapy groups i.e 0.86±0.03. On the other hand values of urea and uric acid were increased significantly i.e 35.97±0.14, 22.94±0.22 respectively (Rey *et al*., 2024).

Liver function markersi.e., alanine transaminase (ALT) with mean ‡ SD 94.1715.43 and aspartate transaminase (AST) with mean ‡ SD 237.8+37.8, showed a marked increase in arsenic-treated rats suggesting liver impairment due to the release of inflammatory cytokines and free radicals as products of arsenic metabolism by liver enzymes. ZnO- Al-NPs nanocomposite caused a significant decrease in alanine transaminase (ALT) with mean ‡ SD 48.50+4.51and aspartate transaminase (AST) with mean ‡ SD 42.8+29.7 levels compared to arsenic-induced hepatocellular carcinoma group. The study was compared with (Ijaz*et al*., 2023).The data shows that the ZnO-*A. Lebbeck* (Al)-NPs nanocomposite (trial drug) had a stabilizing effect on RBC and hemoglobin levels, maintaining them close to those of the negative control, which suggests it does not adversely affect erythropoiesis or hemoglobin synthesis. Additionally, it notably lowered WBC counts compared to the positive control, which could indicate a potential anti-inflammatory or immunomodulatory effect. In contrast, the standard drug Fluorouracil increased hemoglobin but also a moderate increase in WBCs, which could suggest a different mechanism of action or side effect profile in comparison to the trial drug.

To evaluate the surface's smoothness, echogenicity, and nodule presence, ultrasonography was used. A modified score system was used to evaluate the significance of these parameters. The average score of the positive control group was 3.83 = 0.41, which deviated considerably from the mean score of the ZnO-Al-NPs composite treated rats 0.67 # 1.03, which showed the protective effect of the ZnO-Al-NPs composite against HCC (Wei*et al*.,2013).The histological results of this investigation demonstrated that the livers of the

rats treated with arsenic displayed the proliferation of fibroblastic cells, which divided the dysplastic, necrotic and degraded hepatocytes into nodules. ZnO-Al-NP treatment showed histopathological improvement. When Zno-Al-NPs were utilized in treatment.The majority of the histological symptoms seen in cancer control were significantly decreased overall. The outcomes matched those reported by (Medhat *et al*., 2017).

***Conclusion*:** Hence, it was concluded that the ZnO-Al-NPs nanocomposite possesses protective effects against arsenic-induced HCC. It was further confirmed by observing the gross appearance of intact livers of respective rats in each group and by histopathological examination. Biochemical profiles and ultrasonography also suggested that ZnO-Al-NPs nanocomposite possesses either equal or better therapeutic effects than fluorouracil.

***Conflict of Interest*:** The authors do not have any conflict of interest.

***Authors Contribution*:** KH, FH, and MRS Conceptualization, Executed research work, TS, MI, and TMD laboratory work, AM, MB, and KH writing, AR and KI review and analysis, FH supervised the work.

***Acknowledgment*:** We thank the Institute of Physiology and Pharmacology, especially the nanomedicine laboratory, for their support.

REFERENCE

Akbudak, I.H., O. Kilic-Erkek andM. Bor-Kucukatay. 2023. Desflurane 6% inhalation inhibits erythrocyte deformability and alters oxidative stress in rat lung and kidney in a time-dependent manner. Kafkas Universitesi Veteriner Fakultesi Dergisi29:273-279.

Akpinar, D., T. Mercan, H. Demir, S. Ozdemir, C. Demir and S. Kavak. 2023. Protective Effects of Thymoquinone on Doxorubicin-induced Lipid Peroxidation and Antioxidant Enzyme Levels in Rat Peripheral Tissues. Pakistan Veterinary Journal 43:651-658.

Altammar, K.A. 2023. A review on nanoparticles: characteristics, synthesis, applications, and challenges. Frontiers in microbiology 14:1155622.

Anjum, R., M. Hamid, R. Khalil and A. Ajmal. 2023. Possible effect of ascorbic acid against zinc oxide nanoparticles induced hepatotoxicity in Swiss albino mice. International Journal of Agriculture and Biosciences 12:193-198.

Anuk, T., B. Yıldız, R. Demirel, S. İçen M, Yıldırım, H. Beşeren, B. Mor and O. Özden.2022. Usnic acid reduces colon cancer cell viability and colony formation by affecting cancer cell metabolism. Kafkas Universitesi Veteriner Fakultesi Dergisi 28:491-497

Astawibawa, P. O., I.N. Suarsana and I.G.A.A. Suartini. 2022. The influence of peel extract Musa paradisiaca formatypica against kidney histology, ureum levels and creatinin Rattus novergicus after intensive exercise. Buletin Veteriner Udayana 14:578-585.

Azam, S.E., F. Yasmeen, M.S. Rashid and M.F. Latif. 2022. Physical factors affecting the antibacterial activity of Silver (Ag) and Zinc Oxide (ZnO) Nanoparticles (NPs), their application in edible and inedible food packaging, and regulation in food products. International Journal of Agriculture and Biosciences 11:181-193.

Desai, S., and T.C. Taranath. 2022. Characterization and Biological activities of silver nanoparticles synthesized using grewia tiliifolia vahl leaf extract. Pharmaceutical Sciences 29:111-122.

Frase, C.G., E. Buhr and K. Dirscherl. 2007. CD characterization of nanostructures in SEM metrology. Measurement Science and Technology 18:510.

Haryono, A., D.A.A. Nugrahaningsih, D.C.R. Sari, M.M. Romi and N. Arfian. 2018. Reduction of serum uric acid associated with attenuation of renal injury, inflammation and macrophages M1/M2 ratio in hyperuricemic mice model. Kobe Journal of Medical Sciences 64:E107.

Ijaz, M.U., Z. Rafi, A. Hamza, M. Tariq, S. Alkahtani, A.A. Alkahtane and M.N. Riaz. 2023. Tectochrysin Attenuates Cisplatin-induced Hepatotoxicity by Restoring Biochemical, Inflammatory and Histological Profile in Rats. Pakistan Veterinary Journal 43:366-370.

Jayachandran, A., T.R. Aswathy and A.S. Nair. 2021. Green synthesis and characterization of zinc oxide nanoparticles using Cayratia pedata leaf extract. Biochemistry and Biophysics Reports 26:100995.

Kamble, S., S. Agrawal, S. Cherumukkil, V. Sharma, R.V. Jasra and P. Munshi. 2022. Revisiting zeta potential, the key feature of interfacial phenomena, with applications and recent advancements. ChemistrySelect 7:e202103084.

Liang-liang, L., L. Kuan-hui, X. Shi-hui, L. Ting, Z. Guang-shuang, M.H. Almutairi, M. Zahid, M.A. Gondal, Qudratullah, K. Mehmood and Y. Wu. 2023. Immune enhancing activity of garlic polysaccharide, selenizing Codonopsis pilosula compounds in chickens. Asian Journal of Agriculture and Biology 2023:2023149.

Malik, I.R., S. Nayyab, M.R. Mirza, S. Muzammil, J.S. Cheema, K. Imran, K. Nisar, S. Hayat and M. Javed. 2022. Growth performance of major carps during exposure of zinc and bioaccumulation in fish body organs. Asian Journal of Agriculture and Biology2022:202102092.

Mansour MK, Yousif HM, Rezk RAESA, El Mahdy AM 2023. Promotives of nano-zinc oxide as an immune stimulant in the treatment of lambs suffering from zinc deficiency. Kafkas Univ Vet Fak Derg, 29 (2):183-190. DOI: 10.9775/kvfd.2022.28922

Medhat, A., S. Mansour, S. El-Sonbaty, E. Kandil, and M. Mahmoud. 2017. Evaluation of the antitumor activity of platinum nanoparticles in the treatment of hepatocellular carcinoma induced in rats. Tumor biology 39:1010428317717259.

Motta, B.M., M. Masarone, P. Torre and M. Persico. 2023. From non-alcoholic steatohepatitis (NASH) to hepatocellular carcinoma (HCC): Epidemiology, incidence, predictions, risk factors, and prevention. Cancers 15:5458.

Naser, H., S.M. Mohammad, H.M. Shanshool, Z. Hassan and S. Rajamanickam. 2024. Evaluation and Analyzing the Linear Optical Properties of Polymer/Al-Ag-ZnO Nanocomposites. Plasmonics 13:1-16.

Noté, O.P., D. Jihu, C. Antheaume, M. Zeniou, D.E. Pegnyemb, D. Guillaume and A.Lobstein. 2015. Triterpenoid saponins from *Albizia lebbeck* (L.) Benth and their Inhibitory effect on the survival of high grade human brain tumor cells. Carbohydrate research 404:26-33.

Rey, I., R. Effendi-Ys and K. Sukatendel. 2024. The Comparison of Serum Interleukin-8 Levels Based on Severity of Liver Cirrhosis. Medical Archives 78:92.

Samy, A., H.M.A. Hassan and H.M.R. Elsherif. 2022. Effect of nano zinc oxide and traditional zinc (oxide and sulphate) sources on performance, bone characteristics and physiological parameters of broiler chicks. International Journal of Veterinary Science 11:486-492.

Shahid, A., M. Faizan and M.A. Raza. 2023. Potential role of silver nanoparticles (AgNPs) and zinc nanoparticles (ZnNPs) for plant disease management. Agrobiological Records 14: 59-69.

Shaker, N.A., M.H. Hassan, I.A. Emam, Y.H. Ahmed and S.M. EL-Gammal. 2023. Anatomical, histological, and ultrasonographical studies on the liver of red fox (Vulpes vulpes) with its arterial blood distribution andbiliary ducts. International Journal of Veterinary Science 12:324-332.

Sindi, R.A., S. Alam, M. Rizwan, M.I. Ullah, N. Ijaz, Z. Iqbal and R. Hussain. 2023. Investigations of Hemato-Biochemical, Histopathological, Oxidative Stress and Reproductive Effects of Thiram in Albino Rats. Pakistan Veterinary Journal 43:255-261.

Srinivasan, D., A. Premkumar, S. Madanagurusamy and G.S. Natarajamani. 2024. Room temperature detection of isopropyl alcohol using CTAB-assisted silver-doped ZnO nanorods as chemo-resistive gas sensor. Ceramics International 50:39666-77

Umair, M., S. Altaf, H. Muzaffar, A. Iftikhar, A. Ali, N. Batool, T. Iqbal and S.R. Babar. 2022. Green nanotechnology mediated silver and iron oxide nanoparticles: Potential antimicrobials. Agrobiological Records 10:35-41.

Wei, H., J. Huang, J. Yang, X. Zhang, L. Lin, E. Xue and Z. Chen. 2013. Ultrasound exposure improves the targeted therapy effects of galactosylated docetaxel nanoparticles on hepatocellular carcinoma xenografts. PloS one 8:e58133.

Yoo YJ, Lee JH, Yeom DH, Hwang Y, Kim IS, Yoon JC, Tae HJ: Histopathology and tumor necrosis factor-α expression in the kidney of an asphyxial cardiac arrest rat model. Kafkas Univ Vet Fak Derg, 28 (1): 51-58, 2022. DOI: 10.9775/kvfd.2021.26413

Zheng, Y., R. Jia, J. Li, X. Tian and Y. Qian. 2022. Curcumin- and resveratrol-co-loaded nanoparticles in synergistic treatment of hepatocellular carcinoma. Journal of Nanobiotechnology 20:339.

Zhou, X.Q., Z. Hayat, D.D. Zhang, M.Y. Li, S. Hu, Q. Wu and Y. Yuan. 2023. Zinc oxide nanoparticles: synthesis, characterization, modification, and applications in food and agriculture. Processes 11:1193.