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RESEARCH PAPER

The Impact of PtNPs and CDDP on a Rabbit Hepatocellular Carcinoma Model

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ABSTRACT

Hepatocellular carcinoma (HCC), the most common primary liver cancer, accounts for approximately 90% of all liver cancer cases. Asia and sub-Saharan Africa, where hepatitis B infection is common and many people are infected from birth, are the regions with the highest incidence of HCC cases and the lowest post-treatment survival rates. In order to treat rabbits with HCC, this study evaluated the anticancer activity of platinum nanoparticles (PtNPs) to that of cisplatin (CDDP). Measurement of antioxidant activity against diethylnitrosamine (DEN)-induced oxidative stress in liver tissue was used to assess the effectiveness of PtNP therapy. Malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, and reduced glutathione (GSH) content were all measured. In addition to assessing the liver tissue's cytochrome c, caspase-3, and serum alpha-fetoprotein (AFP) levels, liver function tests have been also performed. The relative quantification of the genes for tumor protein p53, matrix metallopeptidase 9, and B-cell lymphoma 2 had also been carried out using complete RNA extraction from biopsies of liver tissue. Since it improved the study's variables to those of typical control animals, the results demonstrated that PtNPs are more effective than CDDP in treating rabbit HCC brought on by DEN. In histological investigations of the DEN group treated with PtNPs, such results were well acknowledged. This suggests, therefore, that PtNPs can act as a promising agent for the treatment of HCC and could therefore interest future investigations.

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INTRODUCTION

Only found in vertebrates, the liver is a significant organ that carries out a variety of crucial biological tasks like detoxifying the body and producing the proteins and biochemicals required for growth and digesting. Furthermore, it is responsible for

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regulating glycogen storage, the breakdown of red blood cells, and the synthesis of hormones. All of these functions are part of the metabolic process [1,2]. The presence of malignant tumors in the liver indicates that the cancer either began in the liver itself or spread there from another part of the body (metastatic liver cancer). The majority of malignant liver tumors are metastatic. Malignant tumors of the liver parenchymal cells are called hepatocellular carcinomas (HCCs). Hepatitis B was identified as the most prevalent cause, with cirrhosis evident in almost 78% of cases. In Iraq and the rest of the world, HCC is ranked fourth and sixth in terms of prevalence [3,4]. Due to an increase in hepatitis C virus infections, the incidence of HCC is rising in the United States and other developing nations. Hepatitis C virus infections are rising, which is contributing to an increase in HCC incidence in the US and other emerging nations. For reasons that are not fully understood, the prevalence of the condition is over four times higher in males than in females. As the primary underlying cause of HCC, hepatitis C virus (HCV) infection or chronic hepatitis B virus (HBV), HCC is frequently linked to chronic liver damage [5–7]. The incidence rate of HCC has doubled over the past few years, and the rate has been rising throughout Iraq [8,9]. A number of environmental and biological factors have been implicated in this [10]. The etiology or course of the illness may also be affected by various factors including tobacco use and exposure to chemicals such as insecticides [11]. A wide range of foods, including cured meat, dried, salted, and smoked fish, soybeans, cheese, ground water with a high nitrate content, and alcoholic beverages contain diethylnitrosamine (DEN) [12,13]. The advancement of putative pre-neoplastic focal lesions is thought to be how DEN, a potent hepatocarcinogen in rabbits, causes HCC without cirrhosis DNA strand breaks, and DNA carcinogen adducts [14–16]. These events influence the increased cell proliferation and hepatocellular necrosis during the early stages of carcinogenesis. Inadvertently identifying cisplatin (CDDP)'s biological activity in 1965 led to its eventual classification as an anticancer medication [17]. The medicinal effect of CDDP is accomplished by interacting with DNA to generate significant lesions known as crosslinks, which block transcription and replication processes and, ultimately, the cell's ability to repair itself, resulting in cellular death [18]. The lack of tumor tissue specificity in CDDP, despite its remarkable therapeutic performance, has some very negative side effects [19]. Multifunctional nanoparticle development has been fueled by breakthroughs in nanotechnology and rising demand in biomedical applications [20–22]. Featuring minimal collateral harm to healthy tissues, nanoparticles have the capability to be the best transporters for delivering treatments, such as anticancer drugs, to afflicted sites [23]. Due to their unique therapeutic, catalytic, and optical capabilities that rely on shape and size, functional platinum nanoparticles (PtNPs) have attracted significant interest in recent years [24]. Nanomaterials made of platinum are particularly well-suited for medicinal purposes. Target-specific routes have been used by functional PtNPs to demonstrate their apoptosis-inducing abilities [25]. The anticancer properties of platinum compounds are particularly strong. This feature is related to the attachment of PtNPs to DNA strands, which is known to hinder DNA replication as well as the mitotic cell division process (Mitosis) [26]. This research sought to examine PtNPs and CDDP's anticancer effects in treating rabbits with HCC that had been artificially produced.

MATERIALS AND METHODS

Like many other nitrosamines, DEN has a very high potential for causing liver cancer in rabbits. Numerous DNA adducts that are mutagenic to various degrees have been discovered. In this research, DEN have been acquired from Wako Chemical Co. (Osaka, Japan). Hydrogen hexachloroplatinate (IV) was obtained from Kojima Chemicals. CDDP was purchased from Nippon Kayaku (Tokyo, Japan) as infusion solution in a 12 mL Cisplatine Mylan vial (1.25 mg/mL). The chosen bacterial strain was inoculated into a 200 mL flask holding a 80 mL sterile nutrient broth to perform PtNP biosynthesis [27]. The flasks were incubated at 25°C on an orbital shaker (180 rpm). Following that, the culture was centrifuged for 8 min at 10000 rpm. In order to develop Pt NPs, the biomass and supernatant were divided and used independently. By combining it with sterilized hexachloroplatinate solution at a final concentration of 0.95 mM, the supernatant was used to evaluate extracellular generation of PtNPs. At room temperature, all of the mixtures of reactions were incubated for 72 hours on a rotating shaker (180 r/min). For the nanoparticles produced using extracellular techniques, further

Fig. 1. Platinum nanoparticle characterization. (a) TEM, (b) UV-visible absorbance spectra of PtNPs.

Group 1 (Control)	Normal and healthy
Group 2 (PtNPs)	Given 10% of the LD_{50} of PtNPs, every five weeks
Group 3 (CDDP)	10% of the LD_{50} of CDDP was intraperitoneally administered for 5 weeks
Group 4 (DEN)	20 mg/kg of DEN was gavaged five times per week for six weeks [30]
Group 5 (DEN+PtNPs)	DEN was administered as in group 4 and, following induction, and as in group 2, PtNPs were administered for a total of five weeks
Group 6 (DEN+CDDP)	DEN was administered as in group 4 and following induction, CDDP was administered for five weeks as in group 3

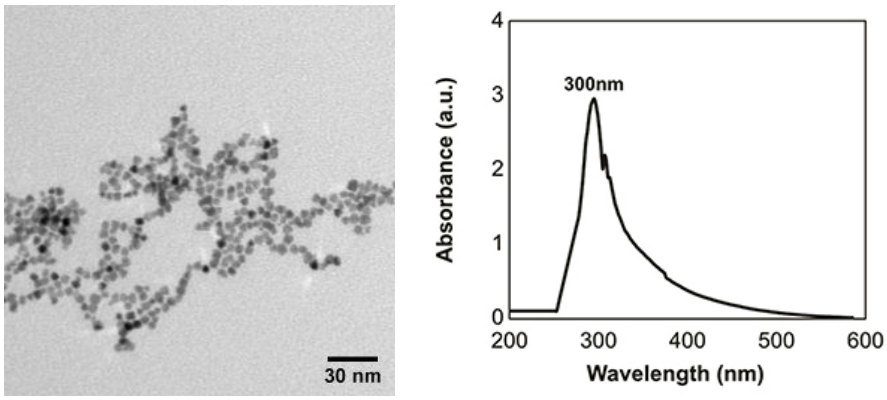
characterization was carried out.

The guide for the care and use of laboratory animals was followed in all animal procedures, which were authorized by University of Baghdad's animal care and use committee. In all, 36 adult New Zealand White (NZW) rabbits weighing 2.5-3.0 kg were used. They were fed a standard rabbit chow and had access to running water during their care. The animals were fed on a regular diet and housed in appropriate cages in a climate-controlled environment (23°C-25°C). The LD_{50} determination is typically the first step in assessing and evaluating a substance's toxic properties when screening drugs [28,29]. For acclimatization, animals were given 14 days. After that, a randomized system was used to divide them into six groups of the same size, each consisting of six rabbits. Table 1 shows are the groups of animals that were identified.

The NZW rabbits were fasted the night before being dissected under a light ether anesthetic at the conclusion of the treatment course. Vena cava blood was collected, centrifuged for 8 min at 2000g.

In liver tissue, lipid peroxidation was assessed [31]. Colorimetric analysis of reduced glutathione content has been performed [32]. Using colorimetric tests, the serum's alkaline phosphatase (ALP), aminotransferases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) activities and total bilirubin, albumin, and total protein level were all measured by diagnostic kits [33]. Alpha Fetoprotein (AFP) ELISA kit acquired from HORIBA, Ltd (Kyoto, Japan) was used to measure AFP levels in serum from each group. Using a rabbit caspase-3 ELISA kit acquired via LifeSpan BioSciences, Inc, USA, the quantity of caspase-3 (CASP3) was measured in liver tissue homogenates from each group. Using a rabbit Cyt c ELISA kit obtained from Abbexa Ltd, United Kingdom, liver tissue homogenates were used to measure the level of cytochrome c (Cyt c).

Utilizing the Qiagen tissue extraction kit (Qiagen, UK), total RNA was extracted. To make sure that there was no DNA contamination in



(a) (b)

Fig. 1. Platinum nanoparticle characterization. (a) TEM, (b) UV-visible absorbance spectra of PtNPs.

the reaction mixture, as a non-template control water was utilized and as an internal control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed. The p53 (tumor protein P53), MMP-9 (matrix metallopeptidase 9), and BCL-2 (B-cell lymphoma 2) complementary DNAs (cDNAs), as well as all other cDNAs, were all made in duplicate.

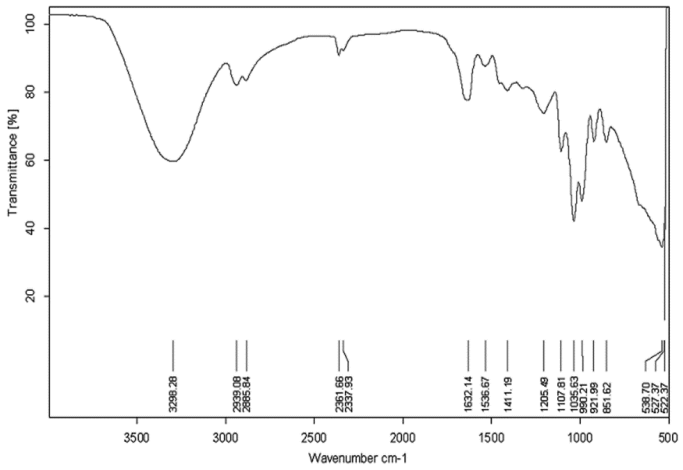
After sacrifice of the animal, its liver tissue was quickly removed, excised, cleaned in a mixture of salt and water, and devide into appropriate pieces before being preserved for 24 hours in 10% neutral buffered formalin (NBF).

One-way analysis of variance (ANOVA) was used to analyze the data. The P values of 0.05 and 0.01 were used as the significant and highly significant level between the mean values. The statistical software SPSS version 23 was used for all calculations.

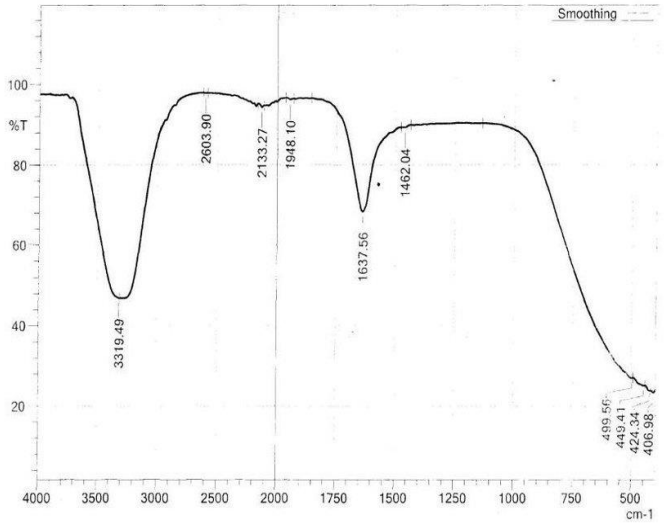
RESULTS AND DISCUSSION

Fourier transform infrared (FTIR), ultraviolet-visible spectroscopy and Transmission electron microscopy (TEM) were used to analyze the produced nanoparticles (Figs. 1 and 2).

The extracellular extract of bacterial broth



(a)



(b)

Fig. 2. The FTIR spectroscopy. (a) bacterial broth's extracellular extract, (b) PtNPs.

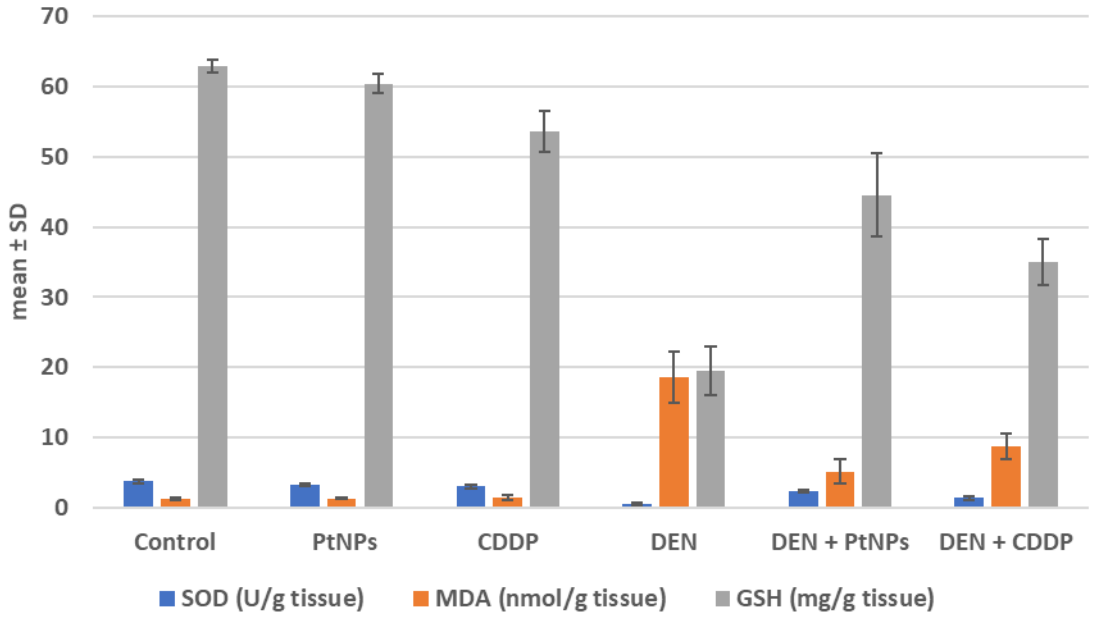


Fig. 3. The levels of liver antioxidants in various groups using ANOVA.

can be seen in the FTIR spectrum to have three prominent intensity peaks: a C single bond N single bond H group at 538.70 cm^{-1}, proteins the amide I at 1632.14 cm^{-1} (due of their high stability and propensity to form resonant structures, amide bonds are frequently found in proteins), and a hydroxyl group at 3298.28 cm^{-1}. The FTIR of PtNPs have also displayed three prominent intensity peaks: a hydroxyl group at 3319.49 cm^{-1}, protein amide I bonds at 1637.56 cm^{-1}, and a C

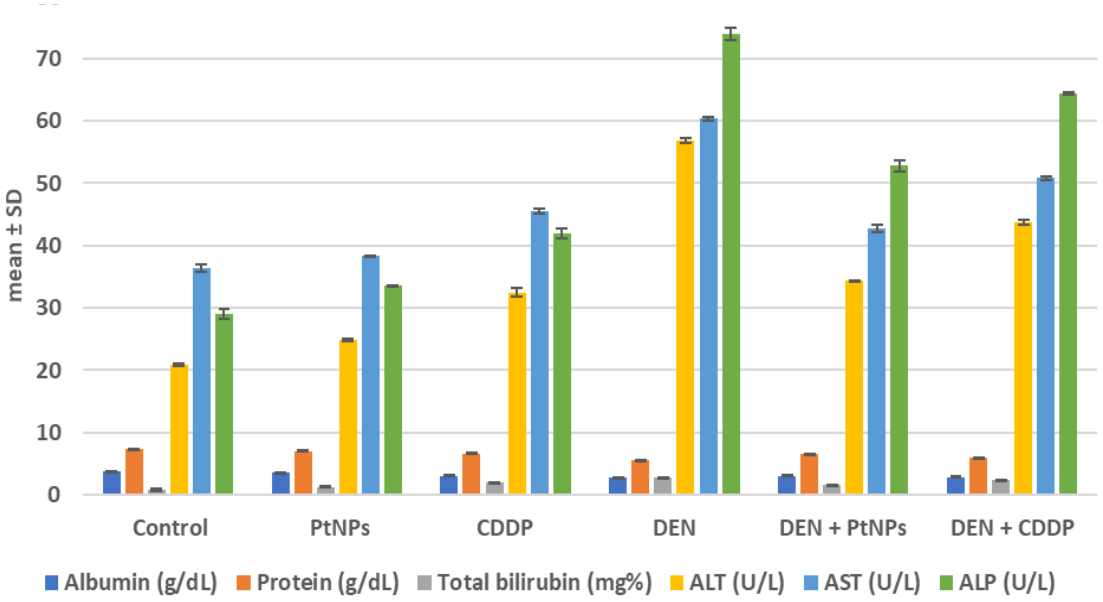


Fig. 4. Tests of liver function in various groups using ANOVA.

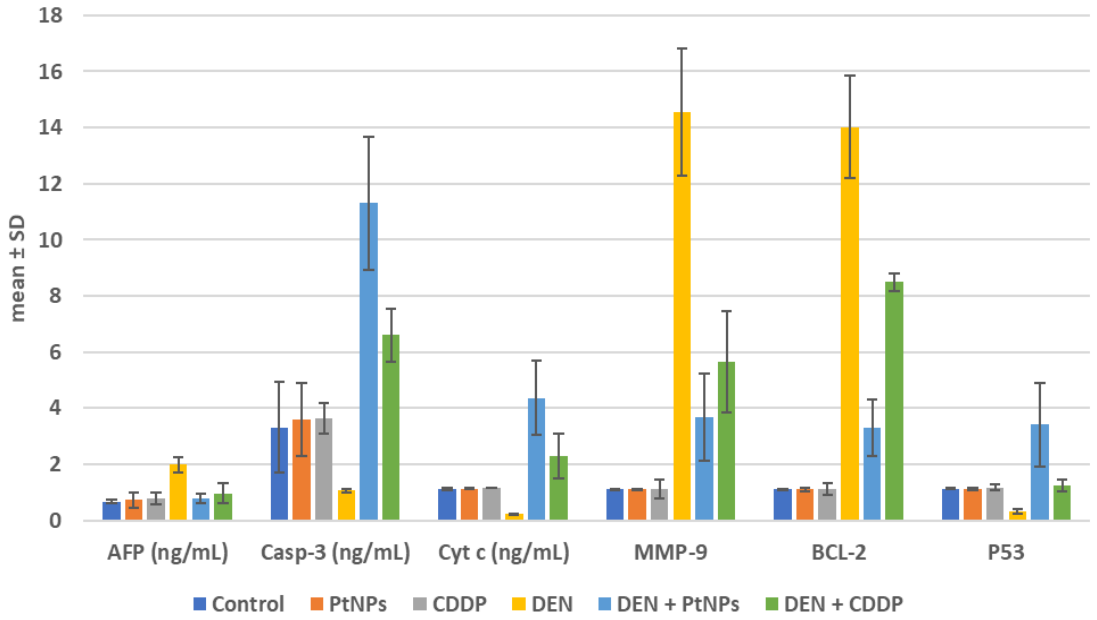


Fig. 5. Relative quantification of p53, BCL-2, MMP-9, liver Casp-3, Cyt c levels, and serum AFP in the various groups using ANOVA.

single bond N single bond H group at 406.98 cm^{-1}, representing the interactions between Bacillus sp. phytochemicals and nanoparticles. We found shifts in the signal wavenumber per centimeter of the principal peaks when comparing the FTIR spectra of bacterial broth's extracellular extract and the PtNPs, showing reduction of the platinum and the creation of the PtNPs as a reduction in the C-H bond and some R-OH oxidation. As a whole, it is possible to draw the conclusion that the proteins produced by Bacillus sp. adsorb as a layer on top of the generated PtNPs, therefore maintaining the nanoparticles that were shaped through the surface-bound proteins.

According to data that was compiled and provided in Fig. 3, the levels of glutathione (GSH) and superoxide dismutase (SOD) in the liver of DEN rabbits were considerably lower than those of control animals.

In comparison to the DEN group, the DEN groups that were treated with PtNPs and CDDP exhibited a considerable rise in the levels of antioxidants. Malondialdehyde (MDA) levels were found to be significantly elevated in DEN animals compared to those of normal rabbits. MDA levels significantly decreased when treated with PtNPs and CDDP compared to DEN rabbits. When compared to groups treated with CDDP, the DEN group treated with PtNPs showed a more substantial rise in levels of GSH and SOD along with a decrease in MDA level.

DEN animals' protein and albumin levels were significantly lower than those of the control group, as demonstrated in Fig. 4.

The findings presented in Fig. 4 revealed that DEN rabbits had significantly higher levels of total bilirubin, as well as ALP, AST, and ALT activity, in comparison to normal rabbits. The results of the relative quantification of P53, BCL-2, and MMP-9 in liver tissue are presented in Fig. 5, together with the liver Casp-3, and Cyt c levels, and serum AFP levels.

When compared to control animals, serum AFP levels were shown to have significantly increased in DEN rabbits. When PtNPs and CDDP were administered to the DEN-induced group, the level of AFP significantly decreased. Comparing DEN model rabbits to normal rabbits, the data revealed a substantial drop in the level of casp-3. In comparison to the DEN and control groups, the treatment of DEN animals with PtNPs and CDDP significantly increased the amount of liver casp-3. DEN animals treated with PtNPs had significantly higher levels of casp-3 than those treated with CDDP. Additionally, the results showed that DEN rabbits had significantly lower liver Cyt C levels than the control group. PtNPs and CDDP therapy for DEN animals exhibited a considerable rise in

Cyt c level when compared to control and DEN rabbits. When compared to DEN animals given CDDP, the PtNPs-subject group likewise showed a highly significant rise in level of Cyt c.

Data showed that DEN animals treated with CDDP and PtNPs had significantly higher levels of liver MMP-9 gene expression than did control rabbits. When DEN animals received various treatments than the DEN group, in the levels of MMP-9 a highly significant drop has been noted. The administration of PtNPs to DEN rabbits resulted in a considerable reduction in MMP-9 levels, which was not the case when DEN animals were given CDDP. Comparing DEN group and DEN animals given PtNPs and CDDP to normal rabbits, level of BCL-2 in liver tissue showed a considerable rise. Level of BCL-2 significantly decreased in the DEN group after receiving various treatments compared to control animals. Rabbits treated with PtNP compared to DEN rabbits given CDDP showed a highly significant reduction in liver BCL-2 levels. When compared to normal rabbits, liver P53 gene expression levels in DEN animals significantly decreased, but in rabbits treated with PtNP, DEN significantly increased. The P53 level was significantly higher in DEN rabbits treated with PtNP and CDDP. Compared to the DEN group that received CDDP, DEN animals exposed to PtNPs displayed a significantly higher level of P53 expression.

It has been suggested that PtNPs could be effective as therapies in the treatment of cancer. Furthermore, the use of PtNPs in conjunction with hadron treatment resulted in an increase of very deadly DNA damage produced by double-strand breaks [34]. In the current investigation, the administration of DEN to NZW rabbits caused large and detrimental shifts in the antioxidant status of the animals. When compared to the group that served as a control, the research showed that there was a considerable increase in the level of MDA in liver tissue along with a marked decrease in the GSH content and SOD activity of liver tissue. These results concurred with those of prior research [35,36]. In DEN rabbits treated with PtNPs and CDDP, the levels of MDA were reduced, while levels of GSH and SOD were elevated, suggesting that these compounds may be effective in inhibiting the intracellular oxidative stress that is brought on by DEN. These findings are consistent with prior findings [37]. Compared to control rabbits, the DEN group had significantly higher levels of total bilirubin and the ALP, AST, and ALT activities. However, total protein and albumin levels were significantly lower in the DEN group than controls which is consistent with prior investigations [38]. Comparing the DEN group to normal animals, this study revealed a substantial drop in Casp-3 levels. In addition, the data indicated that DEN rabbits had lower Cyt c levels than the control group. These outcomes align with earlier research [39].

CONCLUSIONS

The PtNPs are more effective than CDDP in treating HCC in rabbits that was brought on by DEN. PtNPs also resulted in an improvement of the assessed parameters toward normal animals. These results point to the potential of PtNPs as an effective medication for the management of HCC, and they could motivate additional investigation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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