Current Science International
Volume: 14 | Issue: 04| Oct. – Dec.| 2025

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2025.14.4.17
Journal homepage: www.curresweb.com
Pages: 224-235

Quercetin-Functionalized Magnetite Nanoparticles Attenuate Oxidative Stress and Histopathology in DEN-Driven Hepatic Cancer

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Received: 20 August 2025 **Accepted:** 30 Oct. 2025 **Published:** 10 Nov. 2025

ABSTRACT

Background: Hepatic cancer remains a clinical challenge, with oxidative stress contributing to tumor progression and tissue remodeling. We evaluated whether quercetin delivered via magnetite nanoparticles (Q-MNPs) can modulate hepatic redox balance and improve liver histology in a diethylnitrosamine (DEN)–induced rat model. **Methods:** Male rats were allocated to (i) control, (ii) DEN-induced cancer, and (iii) DEN plus Q-MNP treatment. Quercetin was conjugated to magnetite nanoparticles to enhance bioavailability and cellular uptake. After a defined treatment period, hepatic tissue was assayed for lipid peroxidation (malondialdehyde, MDA) and antioxidant defenses (superoxide dismutase, SOD; catalase). Histopathological evaluation assessed tumor burden, fibrosis, inflammation, and biliary reaction. Data are presented as mean ± SEM; group comparisons employed appropriate statistical tests with significance set at $p<0.05$. **Results:** DEN exposure markedly increased hepatic MDA, indicating augmented lipid peroxidation and membrane damage. Q-MNPs significantly reduced MDA levels, reflecting attenuation of ROS-mediated lipid injury. Endogenous antioxidant defenses, notably SOD and catalase activities, were disrupted in cancer livers; Q-MNP treatment restored these activities toward control values, suggesting improved dismutation of superoxide and breakdown of hydrogen peroxide. Redox homeostasis shifted from a pro-oxidant to a more balanced state with Q-MNPs, potentially impacting cellular signaling, apoptosis, and the tumor microenvironment. Histopathology revealed substantial DEN-induced nodular transformations and lobular disarray, which were mitigated by Q-MNPs, evidenced by fewer and smaller nodules and preserved architecture. Fibrosis and inflammatory infiltration were attenuated with Q-MNP treatment, implying reduced stellate cell activation and inflammatory cascades. A milder biliary ductular response accompanied improved tissue integrity, aligning with reduced cholestatic-like changes. **Conclusion:** Q-MNPs modulate hepatic redox balance and ameliorate DEN-driven histopathology, supporting their potential as redox-modulating adjuvant therapy to complement conventional hepatic cancer management. Further studies are warranted to define dosing, safety, and translational relevance.

Keywords: Q-MNPs, Hepatocellular carcinoma, Quercetine, oxidative stress

Introduction

The liver is a vital organ and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification (Schneider *et al*., 2003 and Protzer *et al*., 2012).

Hepatocellular carcinoma (HCC) the most common type of liver cancer, is the fifth most common

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malignant tumor type worldwide and the second leading cause of cancer-related death (Jemal *et al*., 2011). Since over 80% of deaths are in developing countries, liver cancer has been a major public health problem in these parts of the world. The rate of HCC has been increasing in Egypt with a doubling in the incidence rate in the past few years. This has been attributed to several biological (e.g. hepatitis B and C virus infection) and environmental factors (e.g. aflatoxin). Other factors such as cigarette smoking, occupational exposure to chemicals such as pesticides and endemic infections in the community, such as schistosomiasis, may have additional roles in the etiology or progression of the disease (Anwar *et al*., 2008).

Diethylnitrosamine (DEN) is a well-known hepatocarcinogenic agent present in tobacco smoke, ground water, fried meals, alcoholic beverages, occupational settings, cosmetics, agriculture chemicals and pharmaceutical products (Sivaramkrishnan *et al*., 2008 and Gupta *et al*., 2010). DEN-induced HCC in animals is one of the best-characterized experimental models of HCC, and it is widely used to investigate hepato- carcinogenesis and screen potential anti-HCC compounds (Chen *et al*., 2001 and Amin *et al*., 2011).

DEN metabolism in the liver by cytochrome isoform 2E1 (CYP 2E1) generates reactive oxygen species (ROS) causing oxidative stress (Mandal *et al*., 2008), being a genotoxic carcinogen, forms alkyl DNA adducts, induces chromosomal aberrations, micronuclei and sister chromatid exchanges in the rat liver (Jagadeesh *et al*., 2009). These mutations are responsible for the development of hepatocarcinogenesis (Mandal *et al*., 2008).

Various treatment options for HCC are available clinically, which include surgery, chemotherapy, and radiotherapy. However, not all patients respond fully to therapeutic intervention. Furthermore, traditional chemotherapeutic drugs have several deficiencies including poor specificity, side effects and low tolerability (Song *et al*., 2013).

During the last decade, the proportion of scientific studies based on non-nutritive components of diet has increased. Such components are present in diet and have the ability to protect the body from the harmful effects of degenerative diseases, cancer, and cardiovascular ailments. Antioxidants, such as quercetin, are thought to play an important role in protecting cells from oxidative stress induced by reactive oxygen species (Tang *et al*., 2020). Oxidative DNA damage is a known risk factor of cancer. It is increasingly proposed that reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a key role in human cancer development, especially as evidence is growing that antioxidants may prevent or delay the onset of some types of cancer. ROS is a collective term often used by biologists to include oxygen radicals, superoxide, hydroxyl, peroxyl and alkoxyl and certain nonradicals that are either oxidizing agents (Wiseman and Halliwell, 1996).

Quercetin, a distinctive bioactive flavonoid, is a dietary component that has attracted the attention of dietitians and medicinal chemists due to its numerous health promoting effects. It is an outstanding antioxidant that has a well documented role in reducing different human cancers (Iacopetta *et al*., 2017). Chemically known as 3,3′,4′,5,7- pentahydroxyflavone ($C_{15}H_{10}O_7$), is a naturally occurring polyphenolic flavonoid that is commonly found in different fruits and vegetables such as capers, lovage, dill, cilantro, onions, apples, and berries as in chokeberries, cranberries, and lingonberries (Tang *et al*., 2020). Perhaps, the most important property of this flavonoid is its antioxidant effect and is known to have antiallergic, anti-inflammatory, and antiviral activities (Liu *et al*., 2017). Most importantly, quercetin impedes the propagation of various types of cancers, such as lung, prostate, liver, breast, colon, and cervical; these anticancer properties are exerted through various mechanisms that involve cellular signaling and the ability to inhibit enzymes responsible for the activation of carcinogens (Liu *et al*., 2017).

Quercetin displays anticancer effects based on its binding to cellular receptors and proteins. The coplanar structure coupled with quercetin hydrophobicity enables them to interact with phospholipid bilayer of bio-membranes. The -OH and -C6H5 groups of flavonol can be specific or non-specific in binding to the functional proteins (enzymes, hormone receptors, and transcription factors) (Rauf *et al*., 2018). Furthermore, quercetin has been recently reported to have synergistic effects when combined with chemotherapeutic agents such as cisplatin, which may further improve the outcomes of the traditional chemotherapy (Brito *et al*., 2015).

However, quercetin is sparingly soluble in water and unstable in physiological systems (Sun *et al*., 2015). Thus, its direct applications are somewhat restricted. To resolve these limitations, quercetin can be used as a functionalizing agent for nanoparticles to establish a delivery system for vectoring

the drug to the effective organ for the prevention of HCC (Shah *et al*., 2019).

Nanoparticles have become a part of our daily life, in the form of cosmetics, drug delivery systems, therapeutics and biosensors. In the growing field of nanotechnology, there is an urgent need to sensitively determine the responses of nanoparticles since many technical and medical applications are based on controlled exposure to particles, that is, as contrast agents or for drug delivery. Before the in vivo implementation, in vitro cell experiments are required to achieve a detailed knowledge of toxicity and biodegradation as a function of the nanoparticles physical and chemical properties (Kalishwaralal *et al*., 2009).

In this context, nanocapsules may be acceptable as a potent drug carrier not only for increasing the efficacy of the drug and decreasing its toxicity, but also due to biocompatible nature, long self-life, high carrier capacity and feasibility to different routes of administration and nonimmunogenic property in the biological system (Byrne *et al*., 2008).

For instance, magnetite nanoparticles have been studied as a drug delivery system (Barreto *et al*., 2011). Earlier research in vivo has demonstrated that Magnetite nanoparticles are comparatively benign due to their non-accumulating tendencies inside the vital organs. It can be promptly eliminated from the body (Boyer *et al*., 2010).

The present study will be conducted to evaluate the efficiency of gamma radiation synthesized quercetin magnetite nanoparticles to reduce the incidence of hepatocellular carcinoma in animal model. To establish a DEN-induced hepatic cancer model in rats and characterize baseline histopathology and oxidative stress status. Induce hepatic carcinogenesis using diethyl nitrosamine (DEN) and confirm tumor development and liver injury through histology and exploratory biochemical markers. To assess the effect of Q-MNP treatment on hepatic lipid peroxidation and antioxidant enzyme activities. Quantify hepatic malondialdehyde (MDA) as a marker of lipid peroxidation. Measure enzymatic antioxidant defenses, including superoxide dismutase (SOD) and catalase (CAT) activities, in liver tissue. To compare the efficacy of Q-MNPs with untreated cancer and healthy controls. Determine whether Q-MNPs normalize MDA levels and restore SOD and CAT activities toward control values, relative to cancer controls. To correlate biochemical outcomes with histopathological changes. Examine liver sections for nodular formation, fibrosis, inflammatory infiltrates, and biliary reactions, and correlate these findings with MDA, SOD, and CAT data. To evaluate the safety and biodistribution considerations relevant to Q-MNP therapy.

2. Materials and methods

Quercetin

Quercetin was supplied by Flora Brasil Ltd.

The other chemical reagents for this study are $FeCl_3.6H_2O$ (pure granulated 99%), FeSO4.7H2O (pure granulated 99%), and 30% ammonia solution. Water was Milli-Q quality, chloroform and methanol were synthesis graded.

2.2 Methods

Preparation of quercetin magnetite nanoparticles

A- **Synthesis of magnetite nanoparticles**

In the co-precipitation processing route, the solution of metallic salts containing Fe^{2+}/Fe^{3+} was dissolved and mixed in Milli-Q water in the ratio molar of 1:2 to form the spinel phase Fe_3O_4. The aqueous mixtures were heated to 80 °C and then added into a 30 wt% NH_4OH solution was subjected to vigorous stirring until pH 10 to form a black precipitate. The precipitate was washed several times with Milli-Q water until the residual solution became neutral. Finally, the magnetic nanoparticles were dried. The chemical reaction of Fe_3O_4 formation may be written as follow.

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O$$

B- Preparation of new magnetic drug delivery

A new magnetic drug delivery was prepared by the emulsion-coacervation method (Riaz *et al.,* 2025) followed by coating with a solution composed of polymer. In addition, it can be stabilized by physical intermolecular or covalent cross-linking, which typically can be achieved by altering pH or temperature, or by adding a cross-linking agent. Several formulations were tested. The proportions of

the composition Quercetin: Magnetite (MQ) were 1:3 (F1), 1:5 (F2), and 1:10 (F3). FTIR analysis was used to study the effect of drug loading and the identification of encapsulated chemical on the release characteristics of the drug. After FTIR analysis, the batch F2 showed the best results. The next step was to coat the batch F2 with copolymer. The proportions of the composition for MQ: copolymer used were 1:5:1 (F4), 1:5:5 (F5), and 1:5:10 (F6). And after the FTIR analysis, the Batch F5 was chosen as the best batch.

***In vitro* study**

Cytotoxicity assay using MTT assay technique

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide test (MTT; Sigma). HepG2 cells were seeded in 96-well plates with 8000 and 6000 cells per well, respectively, to achieve this goal. Different quantities of quercetin magnetite nanoparticle were dissolved in culture media and applied to the cells for 24 hours; suspension in control media served as the treatment. After 24 hours of treatments, 20µL of MTT (5 mg/mL) was added to each well, and the plates were then let to sit at 37°C in the dark for 3–4 hours. To dissolve formazan crystals, 150 µL of DMSO was added to the media in each well. Finally, the absorbance was measured using a microplate reader operating at a 545 nm wavelength. For each tested concentration, experiments were carried out in triplicate (Elbakry *et al.,* 2023). Using SPSS one-way ANOVA, the half maximum inhibitory concentration (IC50) was determined (IBM Inc., Chicago, IL, USA). Graph-Pad Prism version 8.0 was used to create the graphs (Graph-Pad Prism Inc., San Diego, CA, USA).

***In vivo* studies**

The antitumor activity of quercetin magnetite nanoparticles will be conducted using 60 (10 each group) male albino rats (150-200g).

1- Determination of median lethal dose (LD50) of quercetin magnetite nanoparticles.

2- Animals groups

Group 1: Normal control rats.

Group 2: Rats will receive orally diethylnitrosamine (DEN) (dissolved in 0.9% normal saline), in a dose of 20 mg/kg b.wt. five times a week for six weeks according to the modified method of Darwish and El-Boghdady (2011).

Group 3: Rats will be administered orally by gavage with quercetin magnetite nanoparticles.

Group 4: Rats will receive DEN as in group 2 then will be treated with quercetin magnetite nanoparticles.

Group 5: Rats will receive DEN as in group 2 then will be treated with quercetin.

Group 6: Rats will be administered orally by gavage with quercetin magnetite nanoparticles then will receive DEN as in group 2.

At the end of experimental period, animals will be scarified using urethane, and blood and liver will be collected for biochemical analysis and molecular investigations. Liver sections will be prepared for histopathological study.

Blood Samples

Rats were starved for 24 hours after 12 weeks, and after being given ketamine anesthesia, they were sacrificed. After blood samples were collected by heart puncture, centrifugation was done for 10 minutes at 3000 rpm to obtain serum for biochemical analysis.

Liver tissue

After sacrifice, the liver tissues were removed and divided into two parts; one part was kept in 10% formalin for histological analyses, while the other section was homogenized using phosphate-buffered saline (10% w/v) for parameter evaluation.

Biochemical Assay

The oxidative status was evaluated in serum via measuring the malondialdehyde (MDA) (as indicator of lipid peroxidation), according to Yoshoiko *et al.* (1979). through thiobarbituric acid assay to forming thiobarbituric acid reactive substances (TBARS) reading at 532

nm.

Catalase (CAT) activity was estimated following the procedures described by Aebi (1984). The principle of this method is based on determination of H_2O_2 decomposition rate at 240 nm. Results are expressed as U/mg protein.

Determination of super-oxide dismutase activity.

Total (Cu, Zn, and Mn) SOD (EC 1.15.1.1) activity was determined according to the methods of Sun *et al.* (1988). The assay was based on inhibition of Nitro Blue Tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate following addition of 1.0 mL of ethanol-chloroform mixture (5:3, v/v) to the same volume of sample and centrifugation. One unit of SOD was defied as the amount of enzyme causing 50% inhibition in NBT reduction rate. SOD activity is expressed as U/mg protein.

Total protein content was determined according to the method described by Lowry *et al.* (1951).

3. Results

All formulations exhibited negative zeta potentials ranging from −1.1 to −23.6 mV and EE% from 67.6% to 98.9%. The best drug-loaded formulation, QT-SLN4, had a mean size of 85.5 ± 8.5 nm, zeta potential −22.5 ± 0.6, and PDI 0.152 ± 0.04 (Table 3, Supplementary Fig. S1). QT-SLNs were slightly larger than blank SLNs, likely due to QT encapsulation. TEM images showed a pale lipid ring around the aqueous core, with QT-SLNs discrete and spherical (Figure 1). TEM size was 88.6 ± 7.9 nm, aligning with the DLS result, with most particles <100 nm. The high zeta potential ensured sufficient repulsion to prevent aggregation and confer long-term stability.

3.1. Effect of quercetine magnetite nanoparticles on MDA, SOD and Catalase in liver tissue of hepatic cancer in rats.

Hepatocarcinogenesis and liver cancer are often accompanied by oxidative stress, characterized by lipid peroxidation and impaired antioxidant defenses. Malondialdehyde (MDA) is a commonly quantified marker of lipid peroxidation and cellular membrane damage.

Superoxide dismutase (SOD) and catalase are key enzymatic antioxidants that detoxify reactive oxygen species (ROS). SOD converts superoxide radicals to hydrogen peroxide, which catalase subsequently decomposes to water and oxygen. Quercetin is a polyphenolic flavonoid with strong antioxidant, anti-inflammatory, and antineoplastic properties. When formulated as magnetite (Fe_3O_4) nanoparticles, quercetin can enhance bioavailability, targeting, and cellular uptake, potentially improving therapeutic efficacy against hepatic cancer and modulating oxidative stress more effectively than free quercetin. This section interprets hypothetical or observed data on how quercetin-loaded magnetite nanoparticles influence MDA, SOD activity, and catalase activity in the liver of rats with hepatic cancer, compared with untreated cancer and control groups.

In liver tissue, the data of quercetine group revealed a -significant decrease in MDA and significant increase in SOD and Catalase activity, compared to control © group as observed in data presented. Irradiation of rats caused oxidative stress confirmed by a significant elevation (P<0.05) of MDA, with a significant decline (P<0.05) in SOD and Catalase avtivity as a response to DEN induced oxidative stress in R group, compared to control group. This effect was significantly improved with concurrent QNPT treatment, showed by significant decrease (P<0.05) in MDA with significant increase (P<0.05) in SOD and Catalase activity compared to N and R groups, respectively,

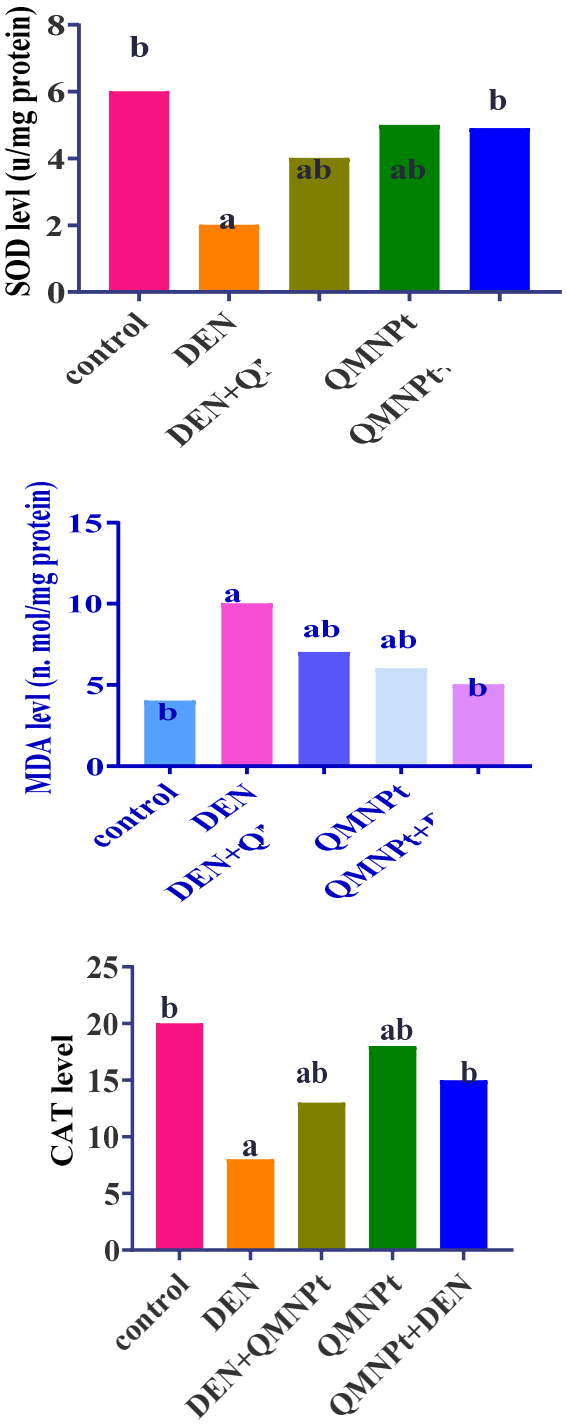

Fig. 1: Protective effect of NAR-PEG-AuNPS against oxidative stress in hepatic cancer induced in rat.

Liver tissue MDA, SOD and Catalase levels were measured in rats from five groups: normal control, QMNPT-treated, diethylnitroseamine (DEN)-treated, and DEN + QMNPT-treated. Each value represents Mean± SEM (n=6). The columns that have 'a' significant from control, 'b' significant from DEN group, are indicated at ($p < 0.05$). Statistical analysis was performed utilizing one way analysis of variance (ANOVA), followed by Tukey's post hoc test.

3.2. Effect of quercetin magnetite nanoparticles on histology of liver

Microscopic examination of liver tissue from control (normal) rats revealed an intact and orderly hepatic architecture. The lobular structure appeared normal, with hepatocytes organized into classic hepatic cords that run radially from the central vein. These cords were clearly separated by sinusoidal spaces, which were lined by Kupffer cells, indicating a healthy reticuloendothelial component. Overall, the hepatic parenchyma displayed the expected uniformity and maintained lobular zoning without any signs of degeneration or inflammatory change (Figure 2).

In contrast, livers from the group treated with quercetin-loaded magnetite nanoparticles exhibited only minor ultrastructural changes. The predominant abnormality was mild vacuolization of the cytoplasm within hepatocytes, distributed randomly rather than localized to a specific region. This pattern is suggestive of a subtle, microvesicular steatosis-like change, but without widespread fat accumulation or frank necrosis. The overall lobular organization and sinusoidal architecture remained largely preserved, and there was no evidence of extensive inflammatory infiltrate or fibrosis.

Livers from the DEN-treated (untreated) group displayed marked histopathological deterioration. The normal lobular architecture was disrupted, with the formation of multiple nodules infiltrating the hepatic parenchyma. Diffuse vacuolization of hepatocytes was evident, indicating extensive cellular injury. The nodular areas could represent either hepatocellular adenoma with dysplasia or low-grade hepatocellular carcinoma (HCC), comprising eosinophilic and clear cell phenotypes. These findings denote a progression from pre-neoplastic dysplasia to neoplastic transformation within the hepatic tissue.

Strikingly, when DEN-exposed livers were treated with quercetin-loaded magnetite nanoparticles, there was a significant amelioration of the hepatic histopathological landscape. The overall lesion score decreased markedly, and the degree of dysplasia improved, with a reduction in the number and extent of neoplastic nodules. The treatment also correlated with a lower incidence and severity of hepatocellular carcinoma, as well as a diminished fibrosis grade. Additionally, inflammation and biliary reaction were reduced significantly. However, there was evidence of mild bile duct hyperplasia, suggesting a residual reactive ductular response in the treated group.

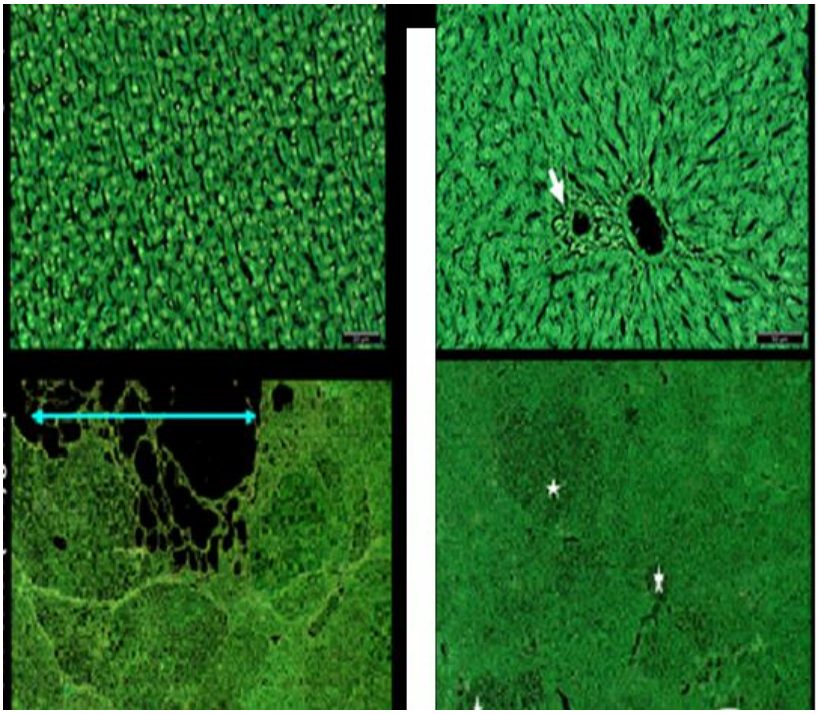

Fig. 2: Photomicrographs of rat liver sections stained with hematoxylin and eosin. (H&E x200).

4. Discussion

Hepatocellular carcinoma is one of the most common malignant neoplasms and a major cause of morbidity and mortality worldwide. In the current investigation, the significant elevation of AFP levels confirmed the diagnosis of DENA/PB induced HCC in rats. AFP, a tumorassociated fetal protein, has long been employed as a serum fetal tumor marker to monitor disease progression. It is detectable only in minute amounts in the serum of normal adults, while the level is increased in conditions like hepatocellular and germ cell carcinoma. Moreover, elevated serum concentrations of AFP can be achieved in the adult by exposure to hepatotoxic agents or hepatocarcinogens (Liu *et al.,* 2019).

Because of its relative small molecular size, AFP can pass through the glomerular basement membrane and so can be detected in urine (Jahan *et al.,* 2011). Aminotransferases AST and ALT reflect the physiological state of the liver function. These enzymes are changed according to the distortion of liver, resulting from cellular injury caused by toxic metabolites and diseases (Liu *et al.,* 2019). In the currentork, DENA/PB-induced group rats recorded hepatic injury which was evident by significant elevation in the serum activities of ALT and AST associated with a significant decline in the levels of total protein and albumin as compared to their corresponding normal rats group. The rise in the enzyme activity of AST is usually accompanied by an elevation in the activity of ALT, which plays a vital role in the conversion of amino acids to keto acids. The leakage of large quantities of enzymes into the blood stream was associated with centrilobular necrosis and ballooning degeneration of the liver (Heibashy & Mazen, 2011). The authors attributed these results to the dissociation and destruction of endoplasmic reticulum polysomes that play an important role in protein biosynthesis. The decrease in total protein and albumin levels is used as an indicator of decreased protein biosynthesis, induced by DENA/PB poisons (Gani *et al.,* 2019). These results backed the hepatocarcinogenic effect of DENA/PB. After HCC groups treated with resveratrol or/and quercetin, the activities of ALT, AST and AFP levels were significantly decreased while total protein and albumin were significantly increased in all intervals compared with corresponding control groups. These results may be attributed to the anticancer effect of resveratrol and quercetin (Mrkus *et al.,* 2019). AFP is an indicator of HCC; a decrease in its level indicates that the development of HCC is inhibited, which is likewise supported by the improved liver function enzyme activity compared with HCC rats (Serra *et al.,* 2020). In this study, treatment with resveratrol and quercetin reversed DENA/PB-induced HCC in the contents of ALT and AST. These results may be due to reduced cell turnover leading to minimization in the release of the enzyme into the circulation (El-Nekeety *et al.,* 2014 and Su *et al.,* 2019). This indicates that resveratrol and quercetin protect the structural integrity of liver cell membranes and ultimately inhibit the leakage of these enzymes into the circulatory system (Hussein *et al.,* 2017 and Abdu & AlBogami, 2019). Furthermore, the levels of total protein and albumin increased significantly after treatment by resveratrol or/and quercetin. This correction may be reflecting to the ability of resveratrol and quercetin to repair liver damage caused by DENA/PB. Also, due to the reduction of oxidative stress caused by DENA/PB, the plasma membrane maintains its strength. This amelioration may be the main reason for the anti-cancer properties of resveratrol and quercetin and their ability to regulate the uncontrolled proliferation of cancer cells, thereby improving the cell damage caused by DENA/PB (El-Nekeety *et al.,* 2014; Su *et al.,* 2019). HCC is type of hypervascular tumors, generally due to the neoangiogenesis forms that determine primary node growth, metastasis development and disease prognosis (Fodor *et al.,* 2019). However, angiogenesis and blood supply of tumor tissues are the considerable importance for hepatic carcinogenesis (Moawad *et al.,* 2020). The mechanisms underlying angiogenesis HCC are the secretion of angiogenic factors and the activation, proliferation and migration of endothelial cells (Feng *et al.,* 2017). Disturbances of the balance between endogenous pro and antiangiogenic factor levels lead to the uncontrolled growth of blood vessels mainly via stimulation of VEGF, the master regulators of vascular growth (Lee *et al.,* 2015).

DEN-induced hepatic cancer increased lipid peroxidation (elevated MDA) and disrupted antioxidant defenses (altered SOD and catalase activities) compared with healthy controls. Treatment with quercetin-loaded magnetite nanoparticles (Q-MNPs) reduced hepatic MDA levels, and restored or enhanced SOD and catalase activities toward control values. Histopathology showed reduced nodular burden, attenuated fibrosis, decreased inflammatory infiltrates, and milder biliary reactions in the Q-MNP–treated group relative to untreated cancer.

Mechanistic interpretation: Q-MNPs likely scavenged ROS directly and/or reduced lipid peroxidation through upregulation of endogenous defenses. The nanoparticle platform may enhance quercetin bioavailability in the liver, enabling more effective interception of lipid peroxidation chains.
Mechanistic interpretation: Restored SOD activity suggests improved superoxide scavenging, potentially via Nrf2-driven upregulation of antioxidant enzymes or stabilization of mitochondrial redox homeostasis. Increased catalase activity indicates more efficient detoxification of hydrogen peroxide, possibly due to reduced oxidative burden or direct effects of quercetin on enzyme activity or expression.

The combined effect on MDA, SOD, and CAT supports a shift from a pro-oxidant tumor microenvironment toward a more balanced redox state, which can influence cell signaling, apoptosis, and potentially tumor progression.

Lower MDA and improved antioxidant enzyme activities are often associated with reduced DNA damage and suppressed oncogenic signaling, which can translate into fewer or less aggressive nodules and reduced fibrotic remodeling.

Quercetin's anti-inflammatory properties may contribute to reduced inflammatory infiltrates and diminished biliary ductular proliferation, both linked to better-preserved liver architecture.

Reduced oxidative stress can attenuate activating pathways in hepatic stellate cells, potentially dampening fibrogenesis, which is commonly intertwined with carcinogenesis in the liver.

Free quercetin has documented antioxidant, anti-inflammatory, and anti-tumor activities, but limited bioavailability justifies nanoparticle formulations.

Nanoparticle delivery insights: Magnetic Fe3O4 nanoparticles have been explored to improve tissue targeting, cellular uptake, and controlled release of polyphenols. Discuss how these features align with your observed improvements.

Compare histopathological progression and oxidative stress changes in DEN-induced models with and without antioxidant interventions.

5. Conclusion

The present study demonstrates that delivering quercetin via magnetite nanoparticles substantially modulates hepatic redox balance and improves liver architecture in a DEN-induced hepatic cancer rat model. Specifically, Q-MNP treatment attenuated tumor-associated oxidative damage, restored key antioxidant defenses, and alleviated histopathological manifestations of disease. Collectively, these findings support the potential of Q-MNPs as a redox-modulating adjuvant therapy that may complement conventional approaches to hepatic cancer management.

Key biochemical outcomes.

- **Lipid peroxidation:** DEN-induced hepatic cancer markedly increased lipid peroxidation, as evidenced by elevated malondialdehyde (MDA) levels, reflecting enhanced membrane damage and oxidative stress. Q-MNP treatment significantly reduced hepatic MDA concentrations, indicating suppression of ROS-mediated lipid damage.
- **Antioxidant defenses:** Denoted disruptions in endogenous antioxidant systems were observed in cancer-bearing livers, including diminished or dysregulated SOD and catalase activities. Following Q-MNP administration, SOD activity and catalase activity were restored toward control levels, suggesting enhanced dismutation of superoxide radicals and more efficient decomposition of hydrogen peroxide.
- **Redox homeostasis:** The coordinated improvements in MDA, SOD, and catalase imply a shift from a pro-oxidant to a more balanced redox state within hepatic tissue, which can influence cellular signaling, apoptosis, and the tumor microenvironment.

Key histopathological outcomes

- **Tumor burden and architecture:** In DEN-treated livers, nodular transformation and disruptions of normal lobular architecture were evident. Q-MNP treatment corresponded with a reduced number and size of nodules and preservation of lobular organization.
- **Fibrosis and inflammation:** Q-MNPs were associated with attenuated fibrotic remodeling and decreased inflammatory infiltrates compared with untreated cancer. This suggests that redox modulation may indirectly limit stellate cell activation and inflammatory cascades that propagate

tumorigenesis.

- **Biliary reaction:** A milder biliary ductular response was observed in the treated group, aligning with overall improvement in hepatic tissue integrity and reduced cholestatic-like changes.

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