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Hepatoprotective Potential of Esculetin Loaded Chitosan Nanoparticles (ESC-CNPs) in DMBA- Induced Mammary Carcinogenesis in Sprague-Dawley Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The hepatoprotective effects of Esculetin Loaded Chitosan Nanoparticles (ESC-CNPs) were assessed in this work using a model of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis. DMBA (25 mg/rat) was injected subcutaneously once close to the mammary gland in Sprague-Dawley rats to cause mammary tumors. To find the ideal dose, several

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ESC-CNPs concentrations were taken orally. The evaluation of hepatoprotective effects involved the examination of liver marker enzymes (AST, ALT and ALP), enzymatic antioxidants (SOD, CAT, and GPx), and non-enzymatic antioxidants GSH, lipid peroxidative markers (TBARS and LOOH), phase I enzymes (NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase) and phase II enzymes GGT and DDT, lipid profile (TC, TG, PL, and FFA) and glycoprotein components (hexose, hexosamine, and sialic acid). The histology of liver tissue was also investigated with PAS staining. According to the results, ESC-CNPs treatment increased phase II detoxification enzyme activity and antioxidant levels in the liver while decreasing levels of liver marker enzymes, lipid peroxidation, phase I detoxification enzymes, lipid profiles and glycoprotein components in comparison to rats treated with DMBA. Furthermore, histopathological investigation validated ESC-CNPs ability to prevent liver damage caused by DMBA. This study employs statistical analysis through Analysis of Variance (ANOVA) to examine the differences between multiple groups in a given dataset.

Keywords: Antioxidant; esculetin; lipid peroxidation; DMBA; hepatocytes; mammary carcinogenesis; chitosan nanoparticles.

1. INTRODUCTION

Breast cancer is the second most common cause of mortality worldwide, and despite a wealth of research and treatment options, its prevalence is rapidly increasing. Vulnerability to this illness is increased by factors including age, hormonal condition, sedentary lifestyles, and continuous direct or indirect exposure to environmental toxins (Rengarajan et al., 2013).

The incomplete combustion of coal, gas, trash, tobacco, and charbroiled meat produces a family of more than 100 compounds known as polycyclic aromatic hydrocarbons, or PAHs. In addition, PAHs are extensively utilized in the manufacturing of insecticides, dyes, plastics, and medications (Anbuselvam et al., 2007). The most well-known PAH and chemical carcinogen among them is 7,12-dimethylbenz(a)anthracene (DMBA), which is commonly used to cause cancer in experimental animals. Enzymes involved in liver detoxification biologically activate the procarcinogen DMBA, resulting in the formation of an ultimate carcinogen (Nandakumar et al., 2011). A major contributing factor to the development of breast cancer is oxidative stress, which is caused by the metabolic activity that produces a lot of free radicals and upsets the oxidant–antioxidant balance in favor of oxidants (Khanzode et al., 2004).

The metabolic activation and detoxification of DMBA take place inside liver cells, despite the liver not usually being considered a main target in DMBA-induced carcinogenesis. Hepatocytes produce reactive oxygen species (ROS) and DMBA-DNA adducts as a result of this mechanism. (Arulkumaran et al., 2007; Ip, and Lisk, 1997). ROS have the ability to seriously harm proteins, lipids, and DNA, which may result in hepatotoxicity and negatively impact hepatocyte function (Arulkumaran et al., 2007; Kumar et al., 2014).

Nanotechnology has become a viable cancer treatment approach in recent years, providing notable benefits over traditional methods (Chehelgerdi et al., 2023). The second most prevalent natural biopolymer, chitosan [(1, 4)-2-amino-2-deoxy-D-glucan], is produced by N-deacetylating chitin. Because it is easily processed into a variety of forms, such as films, threads, beads, membranes, microparticles, and nanoparticles, it is a material that is both plentiful and adaptable. For drug encapsulation, chitosan nanoparticles (CNPs) are especially effective (sankari and subashini, 2023). In order to mitigate DMBA-induced liver damage, this study intends to investigate the potential of CNPs loaded with anticancer drugs as carriers. Bonferoni et al. (2020) reported that targeted drug delivery to the liver using chitosan nanoparticles significantly reduced hepatotoxicity, making it a promising approach for Hepatocellular carcinoma (HCC) therapy.

Esculetin has shown a variety of pharmacological effects include antioxidant, anti-tumor, anti-inflammatory, antibacterial, antidiabetic, immunomodulatory, and anti-atherosclerotic effects (Venugopala et al., 2013; Wang et al., 2015; Arora et al.,2016; Jeon et al., 2016). Citrus limonia, Artemisia capillaris, and Euphorbia lathyris are among the plants that naturally contain it (Cho et al., 2015). Our earlier research showed that ESC-CNPs successfully inhibited

the MDA-MB-231 breast cancer cell line from proliferating in vitro (sankari and subashini, 2023). The anticancer effect of ESC-CNPs in female Sprague-Dawley rats with DMBA -induced breast cancer has yet to be documented.

Investigating the hepatoprotective effects of Esculetin Loaded Chitosan Nanoparticles (ESC-CNPs) in rats with DMBA-induced breast cancer is the goal of this investigation. The study aims to assess the impact of ESC-CNPs on a range of biochemical parameters, such as liver marker enzymes, antioxidant enzymes, lipid peroxidative indicators, biotransformation enzymes, lipid profiles, glycoprotein components, and histopathological alterations in the liver. The results of this experimental model may provide important new information on the wider uses of nanotechnology, highlighting its potential for treating liver damage caused by DMBA.

2. MATERIALS AND METHODS

2.1 Chemicals

Sigma-Aldrich compounds Pvt. Ltd., Bangalore, India, supplied the esculetin and DMBA, whereas all other compounds used in the study were analytical grade.

2.2 Animal Model

Female Sprague-Dawley adult rats weighing 120–130 g and aged 6–7 weeks were acquired from Biogen in Bangalore, India. The animals were kept in the Central Animal House of Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India, with a 12-hour light/dark cycle and controlled environmental conditions at 24 ± 2°C and 50 ± 10% humidity. The rats were given unlimited access to water and standardized rat pellets. Following the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) rules, the Institutional Animal Ethics Committee authorized the experimental procedure (IAEC Proposal No: AU-IAEC 1357/9/23).

2.3 Induction of Mammary Carcinogenesis

Rats were given a single subcutaneous injection of 25 mg of DMBA diluted in a 1 mL emulsion made of physiological saline (0.25 mL) and sunflower oil (0.75 mL) to induce mammary carcinogenesis.

2.4 Experimental Design

The 36 experimental rats were separated into six groups of six rats each.

Group-I Control rats fed standard pellet

Group-II DMBA (25 mg per rat) alone treatment

Group-III ESC-CNPs (100 mg/kg b.w) alone

Group-IV DMBA+ ESC-CNPs (25 mg/kg b.w)

Group-V DMBA+ ESC-CNPs (50 mg/kg b.w)

Group-VI DMBA+ ESC-CNPs (100 mg/kg b.w)

After an overnight fast and an intraperitoneal (I.P.) dose of ketamine hydrochloride to induce anesthesia, the rats were killed by cervical decapitation at the end of the 16th week. As soon as possible, liver tissues were removed, processed, and put in ice-cold containers. On the same day, the tissues were mixed in the suitable buffer, centrifuged for 10 minutes at 3000 g, and the supernatant was utilized for biochemical examination. Furthermore, liver tissues were maintained at -80°C and fixed in 10% formalin for histopathological investigations.

2.5 Preparation of Liver Tissue Homogenate

After washing the removed liver tissues in ice-cold saline, a Potter-Elvehjem homogenizer fitted with a Teflon pestle and running at 600 rpm for 3 minutes was used to create a 10% (w/v) liver homogenate in 0.1 M Tris–HCl buffer (pH 7.4) at 4°C. For biochemical studies, the tissue homogenate was obtained by collecting the supernatant from centrifuging the homogenate at 3000 g for 10 minutes at 4°C. The (Hanioka et al., 1997) approach was used to separate liver microsomes, and the (Lowry et al., 1951) method was used to measure the microsomal protein concentration.

2.6 Biochemical Analysis

2.6.1 Estimation of liver marker enzymes

Bergmeyer et al., 1987 approach was used to quantify the activity of liver marker enzymes, such as alanine transaminase (ALT) and aspartate transaminase (AST). Balasubramanian et al., 1983; King, 1965 described the King technique for estimating alkaline phosphatase (ALP) activity.

2.6.2 Estimation of antioxidant enzymes

The technique of (Kakkar et al., 1984) was used to evaluate the activity of superoxide dismutase (SOD) in liver tissue. Catalase (CAT) activity was measured using the (Sinha, 1972) protocol, and the (Rotruck et al., 1973) method was used to measure glutathione peroxidase (GPx) activity. The Beutler and Kelley et al., 1963 method was used to measure the amounts of reduced glutathione (GSH).

2.6.3 Estimation of lipid peroxidative markers

Lipid hydroperoxide (LOOH) concentrations were ascertained by using the Jiang et al.,1992 methodology, while the levels of thiobarbituric acid-reactive compounds (TBARS) in liver tissue were assessed using the technique outlined by Ohkawa et al., 1979.

2.6.4 Estimation of biotransformation enzymes

The Omura and Takasue technique was used to evaluate the activity of NADPH-cytochrome P450 reductase in liver tissue (Omura and Takasue, 1970). NADH-cytochrome b5 reductase activity was measured, with a few minor adjustments, using the Mihara and Sato, 1972 technique. The Szasz, 1976 technique was used to measure the activity of γ-glutamyl transpeptidase (GGT) in liver tissue [39]. The Ernster, 1967 technique was used, with minor adjustments, to measure the amount of DT-diaphorase (DTD) activity in liver tissue.

2.6.5 Estimation of lipid profile

The Folch et al., (1957) technique was used to extract lipids from liver tissues. The Zlatkis et al., (1953) kit technique was used to quantify the total cholesterol (TC) in liver tissue, and the Foster and Dunn, (1973) method was used to assess the triglycerides (TG). Falholt et al., (1973) approach was used to estimate the amount of free fatty acids (FFA) in liver tissue, whereas Zilversmit and Davis, (1950) method was used to calculate the amount of phospholipids (PL).

2.6.6 Estimation of glycoprotein components

The procedures outlined by Niebes, 1972, Elson and Morgan, 1933, and Warren, 1959 were used to quantify the amounts of hexose, hexosamine, and sialic acid, respectively.

2.6.7 Histopathological studies

The liver samples were dehydrated using a succession of ethanol and water solutions after being originally stored in a 10% formalin solution. Following dehydration, they were imbedded in paraffin and cleaned with xylene. Hematoxylin and eosin were used to stain thin liver slices that were 3-5 micrometers thick. Periodic acid-Schiff staining (PAS) was then used to further stain the specimens. The PAS technique, as outlined by Yamabayashi, was used to measure the amount of glycoprotein in the liver tissues (Yamabayashi, 1987).

2.7 Statistical Analysis

The mean ± SD was used to express the experimental values. One-way analysis of variance (ANOVA) was used to compare mean values between groups, and Duncan's multiple range test (DMRT) was used for multiple comparisons. Statistical significance was considered at $p \leq 0.05$ (p-value).

3. RESULTS

3.1 Effect of ESC-CNPs on Liver Marker Enzymes in the Liver Tissues

Fig. 1 depicts the levels of liver marker enzymes such as AST, ALP, and ALT in liver tissues from control and experimental rats. When rats were treated with DMBA alone, their levels of AST, ALP and ALT were considerably higher than those of control rats. The administration of ESC-CNPs to DMBA-treated rats resulted in drastically reduced liver marker enzymes to near-normal levels. There are no significant differences between the control and ESC-CNPs-alone-treated rats.

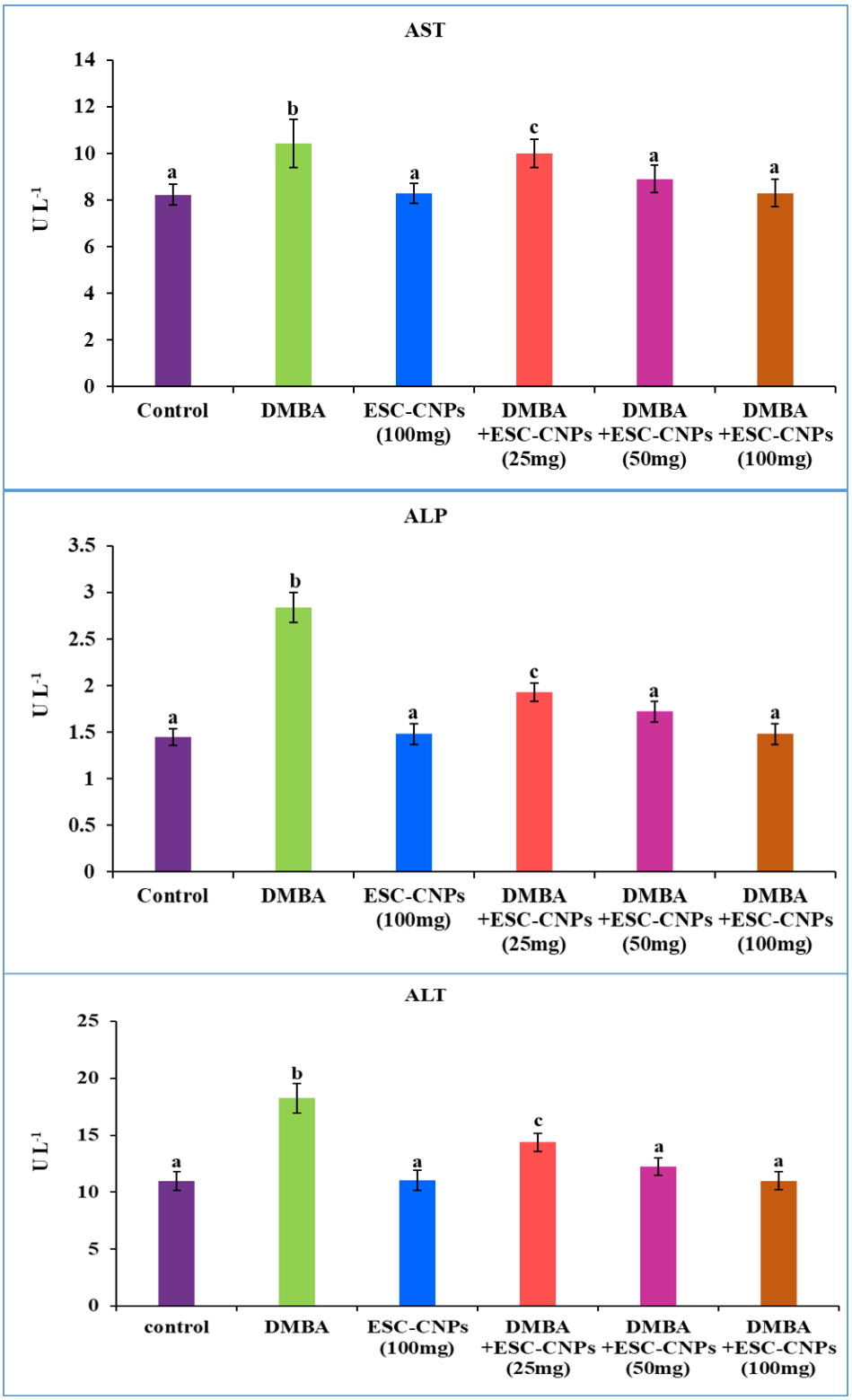

Fig. 1. Effect of ESC-CNPs on liver enzyme in serum of control and experimental rats

Data are expressed as the mean ± SD for six rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p ≤ 0.05 (DMRT). The group I-control; group II-DMBA alone; group III- ESC-CNPs alone (100 mg/kg b.w); group IV- DMBA+ESC-CNPs (25 mg/kg b.w); group V- DMBA+ESC-CNPs (50 mg/kg b.w); group VI-DMBA+ESC-CNPs (100 mg/kg b.w)

3.2 Effect of ESC-CNPs on Antioxidant Status in the Liver Tissues

Fig. 2 depicts the activity of enzymatic antioxidants such as SOD, CAT, and GPx, as well as non-enzymatic antioxidants like GSH, in the liver tissues of control and experimental rats. SOD, CAT, GPx, and GSH levels were considerably lower in DMBA-alone-treated rats compared to control rats. The administration of ESC-CNPs to DMBA-treated rats considerably boosted the activities of both enzymatic and non-enzymatic antioxidants to near-normal levels. There are no significant differences between the control and ESC-CNPs-alone-treated rats.

3.3 Effect of ESC-CNPs on Lipid Peroxidative Markers in the Liver Tissues

The levels of lipid peroxidative markers, including TBARS and LOOH, in the liver tissues of experimental and control rats are displayed in Fig. 3. The tumor-bearing rats had considerably higher levels of LOOH and TBARS than the control rats. The administration of ESC-CNPs into rats treated with DMBA dramatically reduced lipid peroxidative marker levels to near-normal levels. There are no significant differences between the control and ESC-CNPS-alone-treated rats.

3.4 Effect of ESC-CNPs on Detoxification Enzyme Activities in the Liver Tissues

Fig. 4 depicts the activity of phase I enzymes NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase, as well as phase II enzymes GGT and DDT, in liver tissue microsomes from normal and experimental rats. In DMBA-alone-treated rats, the activities of NADPH-cytochrome P450 reductase and NADH cytochrome b5 reductase were dramatically elevated, whereas the activities of GGT and DDT were significantly lowered when compared to control rats. The treatment of ESC-CNPs to DMBA-treated rats considerably reduced phase I enzymes while significantly increasing phase II enzymes to near-normal levels. There are no significant differences between the control and ESC-CNPs-alone-treated rats.

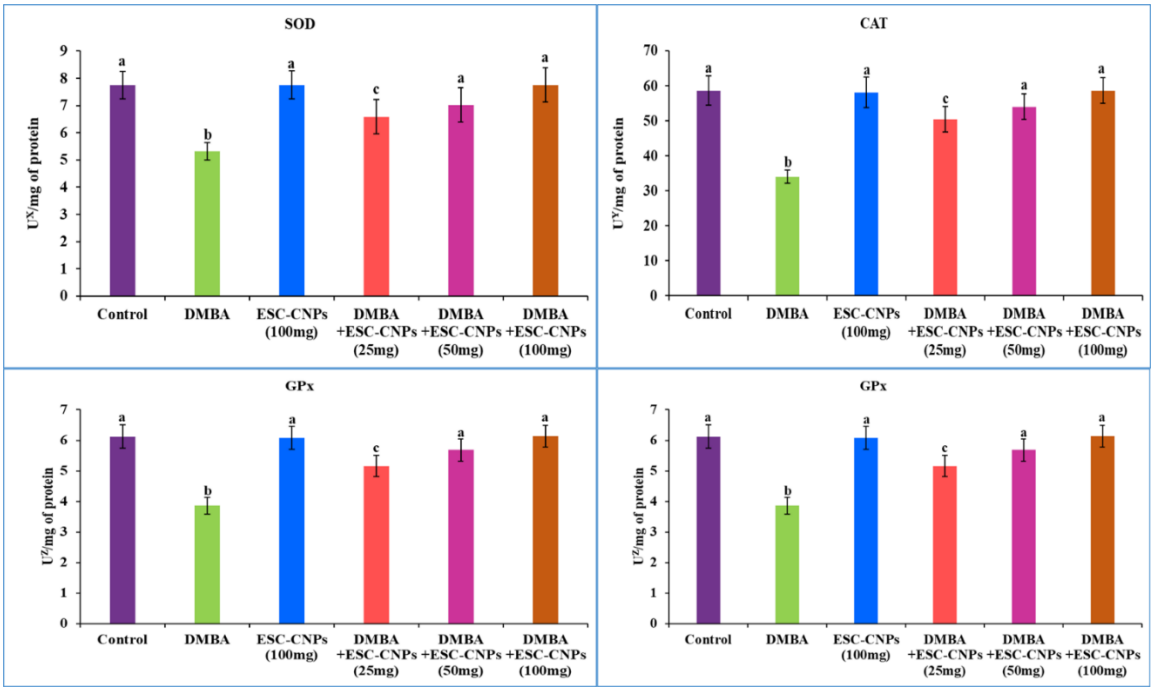

Fig. 2. Effect of ESC-CNPs on antioxidant status in liver tissue of control and experimental rats Data are expressed as the mean ± SD for six rats in each group

Values not sharing a common superscript (a,b,c) differ significantly at p ≤ 0.05 (DMRT). Units for SOD^X, CAT^Y and GPX^Z are expressed as the amount of enzyme required to inhibit 50% of NBT reduction, micromoles of H_2O_2 utilized/second, and micromoles of glutathione utilized/minute, respectively. The group I-control; group II-DMBA alone; group III-ESC-CNPs alone (100 mg/kg b.w); group IV- DMBA+ESC-CNPs (25 mg/kg b.w); group V-DMBA+ESC-CNPs (50 mg/kg b.w); group VI-DMBA+ESC-CNPs (100 mg/kg b.w)

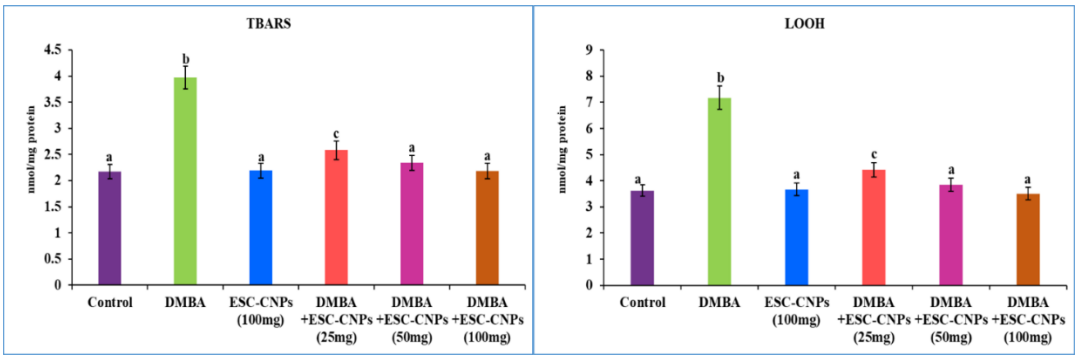

Fig. 3. Effect of ESC-CNPs on lipid peroxidation in liver tissue of control and experimental rats

Data are expressed as the mean ± SD for six rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p ≤ 0.05 (DMRT). The group I-control; group II-DMBA alone; group III- ESC-CNPs alone (100 mg/kg b.w); group IV- DMBA+ESC-CNPs (25 mg/kg b.w); group V- DMBA+ESC-CNPs (50 mg/kg b.w); group VI-DMBA+ESC-CNPs (100 mg/kg b.w)

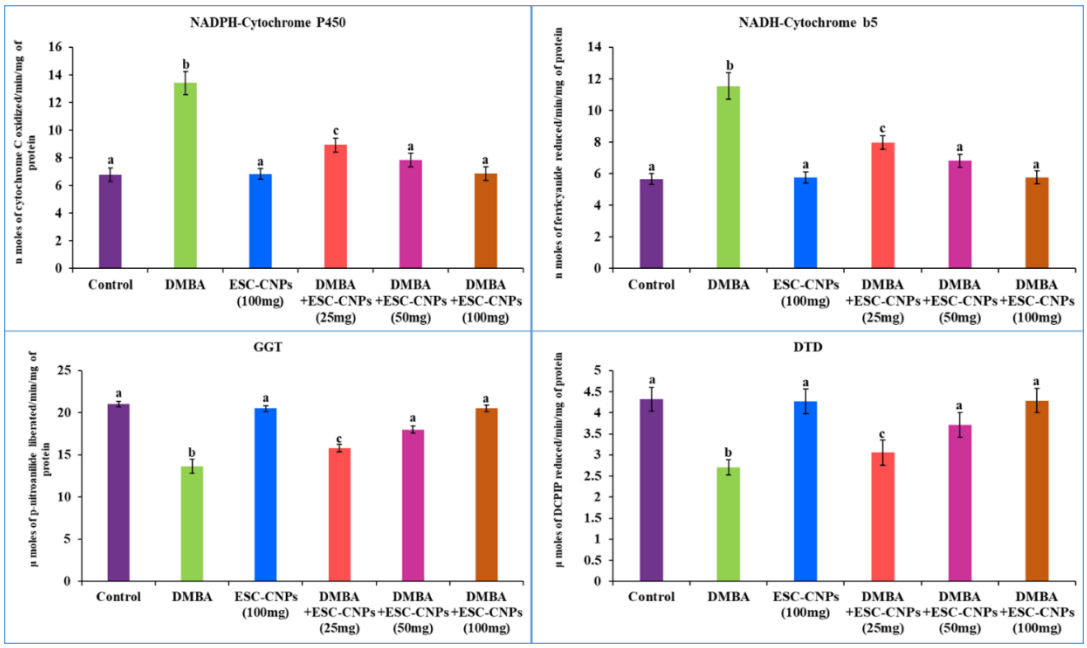

Fig. 4. Effect of ESC-CNPs on detoxification enzymes in liver tissue of control and experimental rats

Data are expressed as the mean ± SD for six rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p ≤ 0.05 (DMRT). The group I-control; group II-DMBA alone; group III-ESC-CNPs alone (100 mg/kg b.w); group IV- DMBA+ESC-CNPs (25 mg/kg b.w); group V- DMBA+ESC-CNPs (50 mg/kg b.w); group VI-DMBA+ESC-CNPs (100 mg/kg b.w)

3.5 Effect of ESC-CNPs on Lipid Profile Levels in the Liver Tissues

Fig. 5 depicts the levels of lipid profile in the liver tissues of control and experimental rats, including TC, TG, PL, and FFA. When compared to control rats, animals treated with DMBA alone had substantially higher levels of TC, TG, PL, and FFA. The treatment of ESC-CNPs to DMBA-treated rats resulted in a lipid profile close to normal. There are no significant differences between control and ESC-CNPs-alone-treated rats.

3.6 Effect of ESC-CNPs on Glycoprotein Component

Fig. 6 shows a comparison of glycoprotein components (hexose, hexosamine, and sialic acid) in liver tissue from normal and experimental

rats. Rats treated alone with DMBA showed significantly higher amounts of these glycoprotein components in the liver. When DMBA-treated rats were given ESC-CNPs orally, their glycoprotein component levels fell and returned to near-normal values. Notably, there were no significant changes in the glycoprotein components of the liver in rats treated with ESC-CNPs or in the control group.

3.7 Histopathological Examination

3.7.1 PAS staining

Fig. 7 shows the accumulation of glycoproteins in the liver tissues of both control and experimental rats. PAS analysis revealed an excess of glycoprotein build-up in the liver tissue of rats treated with DMBA. However, when DMBA-induced mice were given ESC-CNPs (at dosages of 25, 50, and 100 mg/kg b.w.), glycoprotein accumulation was significantly reduced, showing the treatment's anti-neoplastic characteristics.

Based on the biochemical and histopathological data, the optimum dose was determined to be 100 mg/kg body weight, and it was chosen for future investigation due to its considerable liver-protective benefits.

4. DISCUSSION

Breast cancer caused by the chemical carcinogen DMBA has systemic effects, especially on the liver, in addition to affecting the mammary glands (Clemens, 1991). The liver is the third most prevalent location of breast cancer metastasis, accounting for around half of all metastatic breast cancer cases. Among patients, 5-12% develop liver metastases as their major site of cancer recurrence (Bale et al., 2019). The liver, the primary organ involved in the metabolism of DMBA, is severely harmed by the carcinogen's reactive intermediates and oxidative stress. The lack of adequate protection against liver damage provided by current drugs emphasizes the need for safer and more efficient liver function preservation measures (Mishra et al., 2014). Evaluating the hepatoprotective effects of ESC-CNPs in DMBA-induced mammary cancer is the goal of this investigation.

Excessive production of reactive oxygen species (ROS) as a result of the metabolic activation of carcinogens or chemicals via phase I enzymes causes oxidative stress and a biomolecule imbalance. This imbalance promotes lipid peroxidation, mutagenesis, and carcinogenesis (Sener et al., 2007).

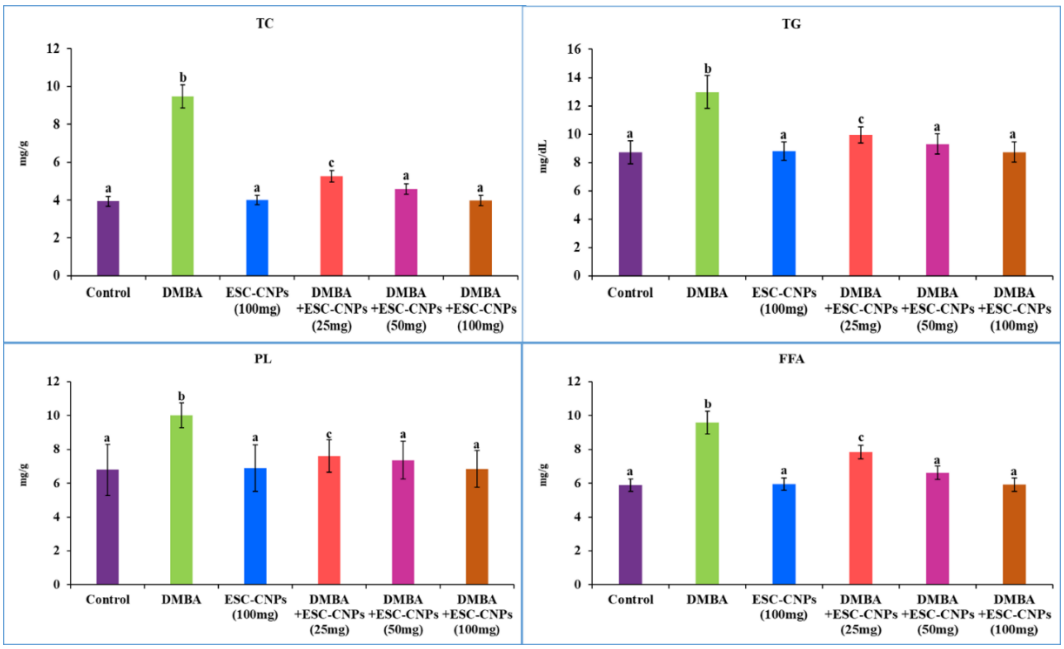

Fig. 5. Effect of ESC-CNPs on lipid profile in liver tissue of control and experimental rats

Data are expressed as the mean ± SD for six rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p ≤ 0.05 (DMRT). The group I-control; group II-DMBA alone; group III-ESC-CNPs alone (100 mg/kg b.w); group IV- DMBA+ESC-CNPs (25 mg/kg b.w); group V- DMBA+ESC-CNPs (50 mg/kg b.w); group VI-DMBA+ESC-CNPs (100 mg/kg b.w)

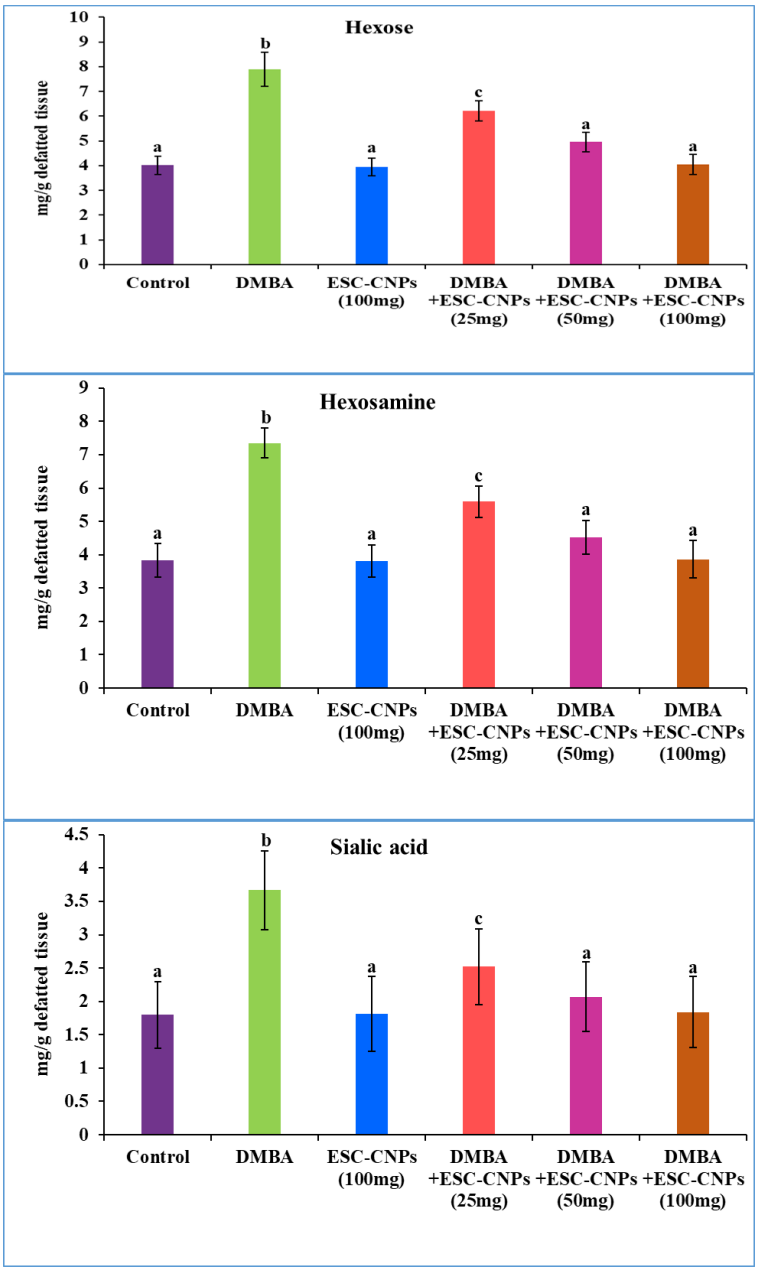

Fig. 6. Effect of ESC-CNPs on glycoprotein components in liver tissue of control and experimental rats

Data are expressed as the mean ± SD for six rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p ≤ 0.05 (DMRT). The group I-control; group II-DMBA alone; group III- ESC-CNPs alone (100 mg/kg b.w); group IV- DMBA+ESC-CNPs (25 mg/kg b.w); group V-DMBA+ESC-CNPs (50 mg/kg b.w); group VI-DMBA+ESC-CNPs (100 mg/kg b.w)

The level of oxidative stress caused by free radicals is determined by detecting lipid peroxidation products in tissues, which indicate cellular damage, disruption of cell integrity, and decreased function (Ramprasath et al., 2006). In rats with DMBA-induced mammary tumors, liver marker enzymes (AST, ALT, and ALP) were shown to be increased in liver tissues. When compared to DMBA rats, oral treatment of ESC-CNPs dramatically lowered levels of key liver marker enzymes (AST, ALT, and ALP). El-Sonbaty et al.,(2022) found that ellagic acid-coated gallium nanoparticles (EA-GaNPs) decreased ALT, AST, and ALP activity in DMBA-induced breast cancer in female Swiss albino rats.

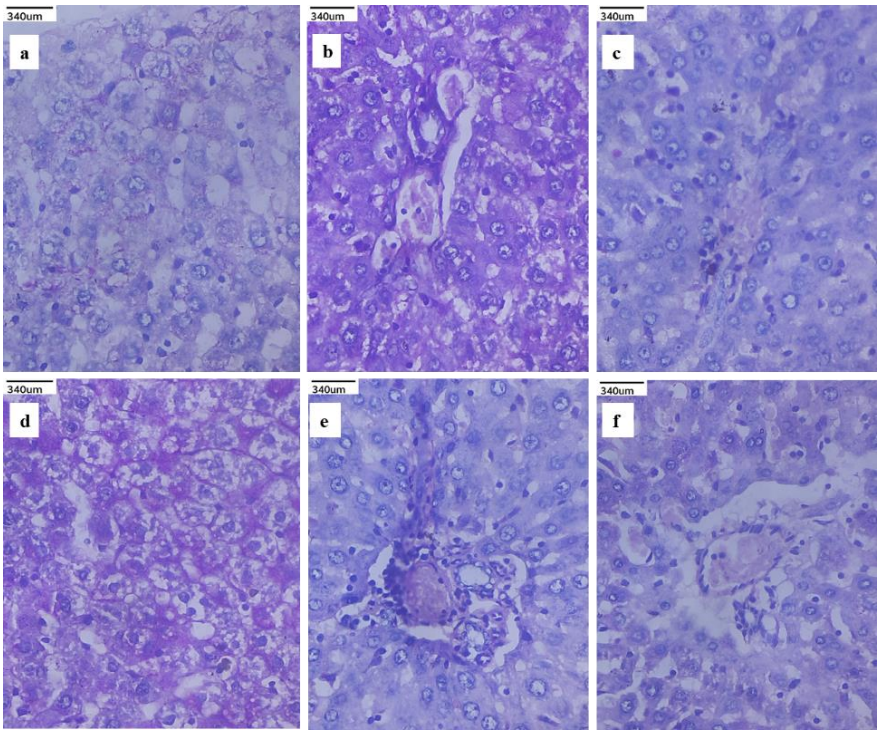

Fig. 7. PAS staining in the liver tissues of control and experimental rats

DMBA alone induced rats (b) showed significantly increased glycoproteins, which were altered in ESC-CNPs treated rats (d, e, f). Controls (a) and ESC-CNPs alone treated rats (c) exhibited very low accumulation of glycoprotein levels. 10X magnification

Antioxidants are necessary to safeguard human health by reducing the detrimental effects of ROS. Both enzymatic and non-enzymatic antioxidants play important roles in scavenging ROS, protecting cells from oxidative DNA damage (Hsu et al., 2011). SOD converts superoxide anion to hydrogen peroxide and molecular oxygen (Kim et al., 2011; Oberley and Buettner, 1979). CAT helps to detoxify hydrogen peroxide by converting it to water and molecular oxygen (Fang et al., 2002). GPx, together with its cofactor GSH, protects cells from oxidative stress by decreasing hydroperoxides (Husain et al., 1987). In the current investigation, SOD, CAT, GSH, and GPx activities were observed to be decreased in SD rats treated with DMBA. However, administering ESC-CNPs greatly boosted the activity of these antioxidants. In a similar context, Vengaimaran et al., (2023) showed that Diosgenin encapsulated in chitosan nanoparticles (DN@CS-NPS) improved antioxidant levels in the liver tissue of DMBA-induced mammary cancer rats. Abd El-Hameed et al., (2021) reported that polydatin-loaded chitosan nanoparticles enhanced antioxidant levels in the liver tissue of nicotinamide/streptozotocin induced diabetic rats.

Tumor cells sequester nutrients and antioxidants from the bloodstream in order to satisfy their dietary requirements and develop rapidly. According to Chang et al., (2011), lipid peroxides produced at the main location might spread to other tissues, perpetuating the lipid peroxidation process. In the current study, TBARS and LOOH activities were observed to be enhanced in SD rats treated with DMBA. The administration of ESC-CNPs lowered TBARS and LOOH activity to near-normal levels. Rajakumar et al., (2018) found that Allyl isothiocyanate decreased the levels of TBARS and LOOH in DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats. Abd El-Hameed et al., (2021) reported that polydatin-loaded chitosan nanoparticles reduced lipid peroxidation levels in the liver tissue of nicotinamide/streptozotocin induced diabetic rats.

The liver detoxifies endogenous, exogenous, and xenobiotic substances and medications (Dasgupta et al., 2003). Liver cells have phase I and phase II detoxification mechanisms that help breakdown and remove harmful compounds from the body. The phase I detoxification process consists of oxidation, reduction, and hydrolysis events mediated by cytochrome P450 enzymes,

the production of which can be triggered by certain substances (Padmavathi et al., 2006). Phase I detoxifying agents transform carcinogens into their final carcinogenic metabolites by metabolically activating them. These metabolites are more easily eliminated through urine when phase II detoxification agents conjugate with reduced glutathione. Treatment with ESC-CNPs restored the impaired Phase I and Phase II detoxification processes in the liver and tumor tissues of tumor-bearing SD rats. In this work, we found an increase in the expression of NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase, as well as a reduction in the expression of GGT and DTD in DMBA-induced rat liver tissues. However, oral treatment of ESC-CNPs to DMBA-treated SD rats regulated detoxifying enzymes, suppressing DMBA-induced mammary carcinogenesis in comparison to control rats. Rajakumar et al., (2018) found that liver tissues of DMBA-induced mammary cancer rats showed elevated phase I enzyme activities and reduced phase II enzyme activity. Mariadoss et al., (2019) discovered that PhCsNPs (phloretin-loaded chitosan nanoparticles) controlled phase I and II enzymes in DMBA-induced oral cancer in hamsters.

Cancer patients frequently have a considerably increased lipid profile, which is most likely owing to cancer cells using lipids as a fuel source for growth and development, as well as altered lipid metabolism associated with the illness (Thangaraju et al., 1994; Nandakumar et al., 2012). Triglycerides (TG) are molecules that store and transport the majority of dietary fats. Fatty acids are required for TG synthesis, which is predominantly utilized to store energy (Santos and Schulze, 2012). Phospholipids (PL) are a kind of lipid composed of a glycerol molecule replaced with one or two fatty acids and an extra polar group (Isabella and Mirunalini, 2017). Free fatty acids (FFA) are abundant throughout the body, with large concentrations in the pancreas and stomach, making them a promising target for treating diabetes and other metabolic diseases (Wu et al., 2017). In the current investigation, total cholesterol (TC), triglycerides (TG), phospholipids (PL), and free fatty acids (FFA) were shown to be elevated in SD rats treated with DMBA. However, oral ESC-CNPs treatment dramatically lowered TC, TG, PL, and FFA levels. According to Rajakumar et al. (2018), AITC lowered TC, TG, PL, and FFA levels in DMBA-induced breast cancer in female Sprague-Dawley rats.

Glycoproteins are essential components of cell membranes, involved in cell adhesion, intracellular protein processing, cell differentiation, signal transduction, host-pathogen interactions, cell activation, and cancer cell metastatic potential (Wang et al., 2008). Glycoproteins include hexose, hexosamine, and sialic acid. Elevated levels of these components in malignant circumstances are strong indicators of the carcinogenic process because they change the structure and function of cell membranes. In this investigation, we found elevated levels of hexose, hexosamine, and sialic acid in tumor-bearing rats treated with DMBA. Veena et al., (2006) found higher glycoprotein component levels in cancer-bearing rats tissues, which was most likely caused by breast tumor connective tissue destruction. Treatment with ESC-CNPs in tumor-bearing rats considerably lowered liver hexose, hexosamine, and sialic acid levels, perhaps due to anti-tumor and anti-metastatic effects. Furthermore, capsaicin-encapsulated chitosan nanoparticles have been demonstrated to reduce levels of liver hexose, hexosamine, and sialic acid in DMBA-induced cancer (Dhamodharan et al., 2021).

PAS (Periodic Acid-Schiff) staining is a histopathological method that detects polysaccharides, such as glycoproteins, on the lipid bilayer surface of cell membranes. Increased PAS staining implies increased glycoprotein content in tumor tissue, which is consistent with prior research by Arivazhagan and Sorimuthu Pillai, (2014). Strong PAS staining intensity is commonly reported in malignant circumstances, suggesting that cancer cells may have increased spreading potential. In this work, DMBA-induced tumor-bearing rats accumulated a substantial amount of glycoprotein. However, when ESC-CNPs were administered, glycoprotein levels were significantly lower than in tumor-bearing rats, indicating that ESC-CNPs may have anti-tumor and anticancer characteristics. Similarly, capsaicin-encapsulated chitosan nanoparticles were found to lower glycoprotein levels in DMBA-induced breast cancer (Dhamodharan et al., 2021).

While this study demonstrates the hepatoprotective effects of ESC-CNPs in DMBA-treated rats, its limitations include reliance on an animal model and the need for detailed mechanistic insights. The findings highlight the therapeutic potential of ESC-CNPs in mitigating hepatotoxicity and suggest broader applications in cancer therapy, particularly in mammary

carcinogenesis. Future research should focus on elucidating underlying mechanisms, performing dose-response studies, and validating these results through clinical trials to assess their applicability in human breast cancer treatment.

5. CONCLUSION

This study finds that ESC-CNPs therapy dramatically reduces liver marker enzymes, lipid peroxidation levels and phase I enzymes and lipid profile and glycoprotein components in the liver tissues of DMBA-treated Sprague-Dawley rats, while increasing antioxidants and phase II detoxification enzymes. Furthermore, histopathological findings confirm ESC-CNPs protective efficacy against DMBA-induced hepatocellular damage in rat liver tissues. Notably, the liver-protective effects of ESC-CNPs were most at 100 mg/kg b.w., indicating a possible therapeutic function in mammary carcinogenesis.

SUPPLEMENTARY DATA

Supplementary data available in this link: https://mbimph.com/index.php/UPJOZ/libraryFiles/downloadPublic/28

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. Quillbot

ETHICAL APPROVAL

This work carried out after getting ethical approval from the Institutional Animal Ethics Committee for the Control and Supervision of Experimental Animals (CPCSEA approval no: 1357).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abd El-Hameed, A. M., Yousef, A. I., Abd El-Twab, S. M., El-Shahawy, A. A., & Abdel-Moneim, A. (2021). Hepatoprotective effects of polydatin-loaded chitosan nanoparticles in diabetic rats: Modulation of glucose metabolism, oxidative stress, and inflammation biomarkers. *Biochemistry (Moscow)*, *86*, 179-189.

Anbuselvam, C., Vijayavel, K., & Balasubramanian, M. P. (2007). Protective effect of *Operculina turpethum* against 7,12-dimethylbenz(a)anthracene-induced oxidative stress with reference to breast cancer in experimental rats. *Chemico-Biological Interactions*, *168*, 229-236.

Arivazhagan, L., & Pillai, S. S. (2014). Tangeretin, a citrus pentamethoxyflavone, exerts cytostatic effect via p53/p21 up-regulation and suppresses metastasis in 7,12-dimethylbenz(\u03b1)anthracene-induced rat mammary carcinoma. *The Journal of Nutritional Biochemistry*, *25*(11), 1140-1153.

Arora, R., Sawney, S., Saini, V., Steffi, C., Tiwari, M., & Saluja, D. (2016). Esculetin induces antiproliferative and apoptotic response in pancreatic cancer cells by directly binding to KEAP1. *Molecular Cancer*, *15*(64), 1-15.

Arulkumaran, S., Ramprasath, V. R., Shanthi, P., & Sachdanandam, P. (2007). Alteration of DMBA-induced oxidative stress by additive action of a modified indigenous preparation—Kalpaamruthaa. *Chemico-Biological Interactions*, *167*(2), 99-106.

Balasubramanian, M. P., & Dhandayuthapani, S. (1983). Comparative studies on phosphomonoesterase in helminthes. *Helminthologia*, *20*, 111-120.

Bale, R., Putzer, D., & Schullian, P. (2019). Local treatment of breast cancer liver metastasis. *Cancers*, *11*(9), 1341.

Bergmeyer, H. U., Schiebe, P., & Wahlefeld, A. W. (1987). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clinical Chemistry*, *24*, 58-73.

Beutler, E., & Kelley, B. M. (1963). The effect of sodium nitrate on RBC glutathione. *Experientia*, *29*, 96-97.

Bonferoni, M. C., Gavini, E., Rassu, G., Maestri, M., & Giunchedi, P. (2020). Chitosan nanoparticles for therapy and theranostics of hepatocellular carcinoma (HCC) and liver-targeting. *Nanomaterials*, *10*(5), 870.

Chang, N. W., Tsai, M. H., Lin, C., Hsu, H. T., Chu, P. Y., Yeh, C. M., & Yeh, K. T. (2011). Fenofibrate exhibits a high potential to suppress the formation of squamous cell carcinoma in an oral-

specific 4-nitroquinoline 1-oxide/arecoline mouse model. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1812*(4), 558-564.

Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q., Pecho, R. D., Jayasankar, N., Rao, D. P., ... & Saadh, M. J. (2023). Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. *Molecular Cancer*, *22*(1), 169.

Cho, J. H., Shin, J. C., Cho, J. J., Choi, Y. H., Shim, J. H., & Chae, J. I. (2015). Esculetin (6,7-dihydroxycoumarin): A potential cancer chemopreventive agent through suppression of Sp1 in oral squamous cancer cells. *International Journal of Oncology*, *46*(1), 265-271.

Clemens, M. R. (1991). Free radicals in chemical carcinogenesis. *Klinische Wochenschrift*, *69*, 1123-1134.

Dasgupta, T., Rao, A. R., & Yadava, P. K. (2003). Modulatory effect of henna leaf (*Lawsonia inermis*) on drug metabolizing phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and forestomach papillomagenesis in mice. *Molecular and Cellular Biochemistry*, *245*, 11-22.

Dhamodharan, K., Vengaimaran, M., & Sankaran, M. (2021). Chemomodulatory effect of capsaicin encapsulated chitosan nanoparticles on lipids, lipoproteins and glycoprotein components in 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary carcinogenesis in Sprague-Dawley rats. *Eurasian Journal of Medicine and Oncology*, *5*(4), 350-357.

Elson, L. A., & Morgan, W. T. (1933). A colorimetric method for the determination of glucosamine and chondrosamine. *Biochemical Journal*, *27*(6), 1824.

El-Sonbaty, S. M., Moawed, F. S., Kandil, E. I., & Tamamm, A. M. (2022). Antitumor and antibacterial efficacy of gallium nanoparticles coated by ellagic acid. *Dose-Response*, *20*(1), 15593258211068998.

Ernster, L. (1967). DT-diaphorase. *Methods in Enzymology*, *10*, 309-317.

Falholt, K., Lund, B., & Falholt, W. (1973). An easy colorimetric micromethod for routine determination of free fatty acids in plasma. *Clinica Chimica Acta*, *46*, 105-111.

Fang, Y. Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, *18*(10), 872-879.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497-509.

Foster, L. B., & Dunn, R. T. (1973). Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clinical Chemistry*, *19*, 338-340.

Hanioka, N., Jinno, H., Nishimura, T., & Ando, M. (1997). Changes in cytochrome P450 enzymes by 1,1-dichloroethylene in rat liver and kidney. *Archives of Toxicology*, *72*, 9-16.

Hsu, C. K., Yeh, J. Y., & Wei, J. H. (2011). Protective effects of the crude extracts from yam (*Dioscorea alata*) peel on tert-butylhydroperoxide-induced oxidative stress in mouse liver cells. *Food Chemistry*, *126*(2), 429-434.

Husain, S. R., Cillard, J., & Cillard, P. (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*, *26*(9), 2489-2491.

Ip, C., & Lisk, D. J. (1997). Modulation of phase I and phase II xenobiotic-metabolizing enzymes by selenium-enriched garlic in rats.

Isabella, S., & Mirunalini, S. (2017). Protective effect of 3,3′-Diindolylmethane encapsulated chitosan nanoparticles prop up with lipid metabolism and biotransformation enzymes against possible mammary cancer. *Journal of Applied Pharmaceutical Science*, *7*(3), 194-201.

Jeon, Y. J., Cho, J. H., Lee, S. Y., Choi, Y. H., Park, H., Jung, S., Shim, J. H., & Chae, J. I. (2016). Esculetin induces apoptosis through EGFR/PI3K/Akt signaling pathway and nucleophosmin relocalization. *Journal of Cellular Biochemistry*, *117*(5), 1210-1221.

Jiang, Z. Y., Hunt, J. V., & Wolff, S. P. (1992). Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein. *Analytical Biochemistry*, *202*, 384-389.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics*, *21*, 130-132.

Khanzode, S. S., Muddeshwar, M. G., Khanzode, S. D., & Dakhale, G. N. (2004). Antioxidant

enzymes and lipid peroxidation in different stages of breast cancer. *Free Radical Research*, *38*, 81-85.

Kim, I. S., Yang, M., Lee, O. H., & Kang, S. N. (2011). The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. *LWT - Food Science and Technology*, *44*(5), 1328-1332.

King, J. (1965). The transferases alanine and aspartate transaminases. In Van D. (Ed.), *Practical and Clinical Enzymology* (pp. 121-138). Van Nostrand Co. Ltd, London.

Kumar, R., Kaur, R., Singh, A. P., & Arora, S. (2014). Diminution of hepatic response to 7,12-dimethylbenz(\u03b1)anthracene by ethyl acetate fraction of *Acacia catechu* Willd. through modulation of xenobiotic and anti-oxidative enzymes in rats. *PLoS ONE, 9*(2), e90083.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Mariadoss, A. V. A., Vinayagam, R., Xu, B., Venkatachalam, K., Sankaran, V., Vijayakumar, S., ... & David, E. (2019). Phloretin-loaded chitosan nanoparticles enhance the antioxidants and apoptotic mechanisms in DMBA-induced experimental carcinogenesis. *Chemico-Biological Interactions, 308*, 11–19.

Mihara, K., & Sato, R. (1972). Partial purification of NADPH-cytochrome b5 reductase from rabbit liver microsomes with detergents, and its properties. *Journal of Biochemistry, 71*, 725–735.

Mishra, S., Aeri, V., & Katare, D. P. (2014). Hepatoprotective medication for liver injury. *Journal of Pharmacy and Pharmaceutical Sciences, 3*, 891–932.

Nandakumar, N., Haribabu, L., Perumal, S., & Balasubramanian, M. P. (2011). Therapeutic effect of hesperidin with reference to biotransformation, lysosomal and mitochondrial TCA cycle enzymes against 7,12-dimethylbenz(a)anthracene-induced experimental mammary cellular carcinoma. *Biomedical Aging Pathology, 1*, 158–168.

Nandakumar, N., Jayaprakash, R., & Balasubramanian, M. P. (2012). Influence of hesperidin on renal cell surface glycoprotein content, nucleic acids, lysosomal enzymes, and macromolecules against 7,12-dimethylbenz[a]anthracene-induced experimental breast carcinoma. *Journal of Experimental Therapeutics and Oncology, 9*, 265–280.

Niebes, P. (1972). Determination of enzymes and degradation products of glycosaminoglycan metabolism in the serum of healthy and varicose subjects. *Clinica Chimica Acta, 42*(2), 399–408.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.

Omura, T., & Takasue, S. (1970). A new method for simultaneous purification of cytochrome b5 and NADH-cytochrome c reductase from rat liver microsomes. *Journal of Biochemistry, 67*, 249–257.

Padmavathi, R., Senthilnathan, P., & Sakthisekaran, D. (2006). Therapeutic effect of propolis and paclitaxel on hepatic phase I and II enzymes and marker enzymes in dimethylbenz(a)anthracene-induced breast cancer in female rats. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 143*(3), 349–354.

Rajakumar, T., Pugalendhi, P., Thilagavathi, S., Ananthakrishnan, D., & Gunasekaran, K. (2018). Allyl isothiocyanate, a potent chemopreventive agent, targets AhR/Nrf2 signaling pathway in chemically induced mammary carcinogenesis. *Molecular and Cellular Biochemistry, 437*, 1–12.

Ramprasath, V. R., Kumar, B. S., Shanthi, P., & Sachdanandam, P. (2006). Therapeutic efficacy of Kalpaamruthaa on lipid peroxidation and antioxidant status in experimental mammary carcinoma in rats. *Journal of Health Science, 52*(6), 748–757.

Rengarajan, T., Nandakumar, N., & Balasubramanian, M. P. (2013). D-Pinitol prevents rat breast carcinogenesis induced by 7,12-dimethylbenz[a]anthracene through inhibition of Bcl-2 and induction of p53, caspase-3 proteins, and modulation of hepatic biotransformation enzymes and antioxidants. *Biomedical Preventive Nutrition, 3*, 31–41.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science, 179*, 588–590.

Sankari, T. S., & Subashini, R. (2023). Synthesis and characterization of esculetin-loaded chitosan nanoparticles (Esc-CNPs) against

breast cancer cell line (MDA-MB-231) in vitro study. *Wutan Huatan Jisuan Jishu, 19*, 8–30.

Santos, C. R., & Schulze, A. (2012). Lipid metabolism in cancer. *FEBS Journal, 279*(15), 2610–2623.

Sener, D. E., Gönenç, A., Akinci, M., & Torun, M. (2007). Lipid peroxidation and total antioxidant status in patients with breast cancer. *Cell Biochemistry and Function, 25*, 377–382.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry, 47*, 389–394.

Szasz, G. (1976). Reaction-rate method for gamma-glutamyl transferase activity in serum. *Clinical Chemistry, 22*, 2051–2055.

Thangaraju, M., Kumar, K., Gandhirajan, R., & Sachdanandam, P. (1994). Effect of tamoxifen on plasma lipids and lipoproteins in postmenopausal women with breast cancer. *Cancer, 73*(3), 659–663.

Veena, K., Shanthi, P., & Sachdanandam, P. (2006). Anticancer effect of Kalpaamruthaa on mammary carcinoma in rats with reference to glycoprotein components, lysosomal and marker enzymes. *Biological and Pharmaceutical Bulletin, 29*(3), 565–569.

Vengaimaran, M., Dhamodharan, K., & Sankaran, M. (2023). Nano diosgenin abates DMBA-induced renal and hepatic toxicities: Biochemical and histopathological evaluation on the breast cancer model. *Current Bioactive Compounds, 19*(4), 47–67.

Venugopala, K. N., Rashmi, V., & Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity. *Biomedical Research International.* https://doi.org/10.1155/2013/963248

Wang, J., Lu, M. L., Dai, H. L., Zhang, S. P., Wang, H. X., & Wei, N. (2015). Esculetin, a coumarin derivative, exerts in vitro and in vivo antiproliferative activity against hepatocellular carcinoma by initiating a mitochondrial-dependent apoptosis pathway. *Brazilian Journal of Medical and Biological Research, 48*(3), 245–253.

Wang, Y., Ao, X., Vuong, H., Konanur, M., Miller, F. R., Goodison, S., & Lubman, D. M. (2008). Membrane glycoproteins associated with breast tumor cell progression identified by a lectin affinity approach. *Journal of Proteome Research, 7*(10), 4313–4325.

Warren, L. (1959). The thiobarbituric acid assay of sialic acids. *Journal of Biological Chemistry, 234*(8), 1971–1975.

Wu, Y., Yu, X., Yi, X., Wu, K., Dwabe, S., Atefi, M., ... & Vadgama, J. V. (2017). Aberrant phosphorylation of SMAD4 Thr277-mediated USP9x–SMAD4 interaction by free fatty acids promotes breast cancer metastasis. *Cancer Research, 77*(6), 1383–1394.

Yamabayashi, S. (1987). Periodic acid–Schiff–Alcian Blue: A method for the differential staining of glycoproteins. *Histochemical Journal, 19*, 565–571.

Zilversmit, D. B., & Davis, A. K. (1950). Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *Journal of Laboratory and Clinical Medicine, 35*, 155–160.

Zlatkis, A., Zak, B., & Boyle, A. J. (1953). A method for the direct determination of serum cholesterol. *Journal of Laboratory and Clinical Medicine, 41*, 486–492.

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