EJMO

DOI: 10.14744/ejmo.2021.61180
EJMO 2021;5(4):350–357

Research Article

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

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Abstract

Objectives: Breast cancer is a dreadful public health issue that kills plenty of women all around the world. The peril of breast cancer is strongly linked to lipids, lipoproteins, and glycoproteins. Capsaicin (CAP), a natural alkaloid isolated from chilies, has been reported to possess excellent anti-cancer activity. Unfortunately, the clinical application of this compound is strictly limited due to its low solubility and poor bioavailability. Nanoparticle-based drug delivery systems have set the path for a revolution in cancer therapy by improving its therapeutic value. The aim of the present study was to investigate the effect of CAP encapsulated chitosan nanoparticles (CAP@CS-NP) on lipids, lipoproteins and glycoproteins abnormalities in 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis.

Methods: A mammary tumor was induced by a single dose of DMBA 25mg/kg b.wt injected subcutaneously near the mammary gland. The levels of lipid profile, lipoproteins and glycoprotein components were analyzed in the plasma, liver and mammary tissues.

Results: We observed higher levels of total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), hexose, hexosamine and sialic acid in DMBA induced tumor-bearing rats. Moreover, low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels were raised and high-density lipoprotein cholesterol (HDL-C) levels were dropped in tumor-bearing rats. The result shows that, CAP@CS-NP 4mg/kg b.wt administration significantly recouped abnormal levels to near-normal levels. It was additionally verified by histological staining in mammary tissues.

Conclusion: Our findings suggest that nanoencapsulation of CAP@CS-NP successfully regulates lipid profile, lipoproteins, and glycoproteins levels.

Keywords: Breast cancer, Capsaicin, Nanoparticle, Glycoproteins, Lipids

Cite This Article: *Dhamodharan K, Vengaimaran M, Sankaran M. 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. EJMO 2021;5(4):350–357.*

Breast cancer is the most frequent disease in women and the main cause of cancer-related mortality, alarming a global public health issue. It accounts for 11.7% of all cancer cases and 6.9% of all cancer deaths worldwide, according to GLOBOCAN 2020.[1] Obesity and high-fat diets are continuously incriminated as major risk factors for breast cancer owing to lifestyle and dietary factors. Several pathways have been proposed as potential mediators of stress's

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Submitted Date: July 31, 2021 **Accepted Date:** October 05, 20221 **Available Online Date:** December 01, 2021

influence on the neoplastic process, including alteration in the lipid metabolism and glycoprotein components status.[2] One of the hallmarks of human cancer is altered lipid metabolism, which may promote tumor formation by offering the energy and membrane building blocks for brisk cancer cell growth.[3,4] Lipids are an extremely complex category of biomolecules that are not only a structural base of biological membranes but also signaling molecules. Glycoproteins are conjugated protein which covalently binds to carbohydrate residues through the process of glycosylation. It is involved in a variety of mechanisms that lead to proliferation, invasion, and metastasis.[5] Buildout of mammary tumors warrants anomalous aggregation of cells provoked by inordinate proliferation, deficient apoptosis and dysregulation of cellular division out of which 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma is well known.[6]

The chemomodulatory effect of compounds is deemed as one of the effective ways to stop cancer progression and metastasis. It is defined as the administration of chemical agents, either as specific drugs or as naturally available components to alleviate cancer. Recent laboratory studies and epidemiological evidence have also proven that certain pharmacologically active compounds spotted in the diet may diminish the risk of cancer development.[7,8] Capsaicin (8-methyl-N-vanillyl-6-noneamide) (CAP) is a chemical that originates in the chili peppers of the genus Capsicum, represents a crucial ingredient in spicy foods consumed all around the world. Based on a handful of publications, CAP exhibited a powerful anti-carcinogenic effect in a variety of cancers.[9-13] A major obstacle in the clinical practice of CAP is low aqueous solubility and limited bioavailability, which can lead to weakened therapeutic efficacy. So, new strategies are required to boost the solubility and bioavailability of effective drugs.

In recent decades, nanotechnology has popped up as a speedily burgeoning and implemented technology in various fields, including biosensors, electronics, and biomedicine due to its special nanoscale structure and high surface-to-volume ratio tunable features.[14-16] The utilization of nanotechnology to medical and pharmaceutical formulations typically alluded to as nanomedicine is modernizing the medical sector by offering more optimal therapeutics, medical devices, and diagnostics. Encapsulation of phytochemicals in nanoparticles has been proved as a feasible strategy to enhance the aqueous solubility and bioavailability of highly hydrophobic drugs.[17] N-deacetylation of chitin produces chitosan (CS), a biocompatible, bioadhesive, biodegradable and nontoxic linear amino polysaccharide. It has a lot of potential in food, environmental, and pharmaceutical applications because of its unique physiochemical qualities. CS formulations with a high level of deacetylation are recommended in drug delivery systems because of their greater degradation rate.[18]

Hence, with this backdrop, the present investigation has been carried out to study the chemomodulatory effect of CAP encapsulated CS nanoparticles (CAP@CS-NP) on alterations in the Total cholesterol (TC), Triglycerides (TG), Phospholipids (PL), Free fatty acids (FFA), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), Very low-density lipoprotein cholesterol (VLDL-C), hexose, hexosamine and sialic acid during DMBA-induced mammary carcinogenesis in female Sprague–Dawley rats.

Methods

Chemicals

CAP, DMBA, CS, sodium tripolyphosphate (TPP), and all other chemicals were purchased from Sigma-Aldrich Co.Ltd. Reagent kits for TC, TG, and HDL-C were purchased from Agappe Diagnostics, Ernakulam, India.

Preparation and Characterization of CAP@CS-NP

CAP@CS-NP was synthesized by a novel method of ionic gelation with TPP solution (Gelling agent) and characterized by UV–visible spectroscopy, SEM analysis, FT-IR analysis, and In vitro drug release.[19]

Rats and Diet

Female Sprague-Dawley rats aged 8 to 10 weeks old (130 to 150g) were purchased from Biogen Laboratory Animal Facility, Bangalore, India. Rats were maintained on a standard pellet diet (composition of 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorous, 3.4% glucose, 2% vitamins and 55% nitrogen free extract) and were provided feed and water ad libitum. Each polypropylene cage houses six rats which are acclimatized to laboratory conditions with temperature 24±2 °C, humidity 50±10%, and photoperiod of 12 h (dark/light cycle). This study was approved by the Institutional Animal Ethics Committee (IAEC), regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Reg No. 160/1999/CPCSEA and Proposal No. 1203).

Tumor Induction

DMBA was used as a carcinogen for the present investigation. Mammary cancer was induced according to the method of Chidambaram and Baradarajan.[20] A single dose of 25 mg/kg b.wt of DMBA diluted in 1 mL emulsion of sunflower oil (0.75 mL), and physiological saline (0.25 mL) was given

subcutaneous injection near the mammary gland to induce mammary tumor.

Dose Selection

Recent literature studies have proven CAP is effective in chronic inflammation and metabolic disorder. Anandakumar et al. suggest that CAP has the possible anti-cancer potential by the dose of 10mg/kg b.wt in Swiss albino mice model.[21] Based on this literature study, we decided two doses, CAP 8mg/kg b.wt and CAP@CS-NP 4mg/kg b.wt. These doses are administered orally to tumor-bearing rats (After 7 weeks of tumor induction).

Experimental Design

A total number of 36 rats were randomized into six groups, and each group contained six rats. Group I rats served as control (normal untreated rat). Groups II, III, IV & V rats were received 25 mg/kg b.wt of DMBA during the first week of the experiment. Group II rats received no other treatment, and groups III, IV & V rats were treated with different doses of CAP, CAP@CS-NP, and CS-NP (8, 4, and 5 mg/kg b.wt) respectively for 21 days (three times a week). Group VI rats received bare Free CAP@NP for 21 days (three times a week) and served as drug control. The experiment was terminated at the end of the 14th week, all the rats were sacrificed. Blood samples were collected in a heparinized tube, and plasma was separated for the assays. Mammary and liver tissues were separated without delay and washed well with ice-cold saline and homogenized in Tris–HCL buffer (0.1 M, pH 7.4), and used for further analysis.

Estimation of Lipids and Lipoproteins

The lipids were extracted and quantified by the method of Folch et al.[22] TC in plasma, liver, and mammary tissues were estimated by using the kit method of Zlatkis et al.[23] TG in plasma, liver, and mammary tissues were measured by the method of Foster and Dunn.[24] PL in plasma, liver, and mammary tissues were determined by the method of Zilversmit and Davis.[25] FFA in plasma, liver, and mammary tissues were estimated by the method of Falholt et al.[26]

HDL-C in plasma was estimated by the method of Wilson and Spiger using a reagent kit.[27] LDL-C and VLDL-C in plasma were calculated by Friedwald et al.[28] LDL-C = TC – (HDL-C + VLDL-C), VLDL-C= TG/5.

Estimation of Glycoproteins

Hexose in plasma, liver, and mammary tissues were estimated by the method of Niebes.[29] Hexosamine in plasma, liver, and mammary tissues were estimated by the method of Elson and Morgan.[30] Sialic acid in plasma, liver, and mammary tissues were estimated by the method of Warren.[31]

Histopathological Analysis

Sample of the mammary tissue in every group was sliced, immersed in 10% neutral buffered formalin for fixation, dehydrated with graded ethanol solutions, and then embedded in paraffin. Paraffin-embedded mammary tissue sections (3–5 µm) were cut using a microtome. Glycoprotein content in the mammary tissues was analyzed by Periodic Acid Schiff (PAS) base staining according to the method of Yamabayashi.[32]

Statistical Analysis

The data were expressed as mean±standard deviation (SD). Statistical analysis was carried out using IBM SPSS Statistics for Windows, version23 (IBM Corp., Armonk, N.Y., USA). The comparisons between groups were done using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A value of $p<0.05$ was considered statistically significant.

Results

Effect of CAP and CAP@CS-NP on Lipid Profile in Plasma, Liver and Mammary Tissues

Figure 1, 2 and 3 shows the levels of lipids (TC, TG, PL, and FFA) in plasma, liver and mammary tissues of control and experimental rats, respectively. The levels of TC, TG, PL, and FFA were significantly ($p<0.001$) increased in DMBA induced rats (Group II) when compared with control rats (Group I). On the flip side, administration of CAP 8mg/kg b.wt (Group III) and CAP@CS-NP 4mg/kg b.wt (Group IV) significantly curtailed the levels of TC, TG, PL, and FFA when compared with DMBA induced rats (Group II). However, no significant

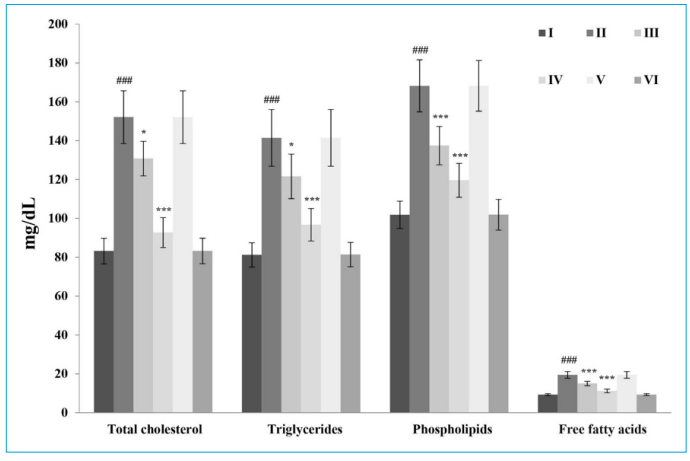

Figure 1. Effect of CAP and CAP@CS-NP on lipid profile in plasma of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###$p<0.001$ when compared with control group and *$p<0.05$, ***$p<0.001$ when compared with DMBA group.

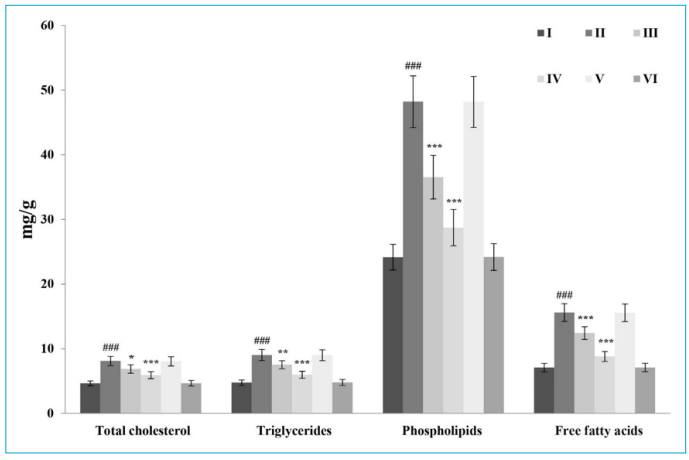

Figure 2. Effect of CAP and CAP@CS-NP on lipid profile in liver tissue of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###p<0.001 when compared with control group and *p<0.05, **p<0.01, ***p<0.001 when compared with DMBA group.

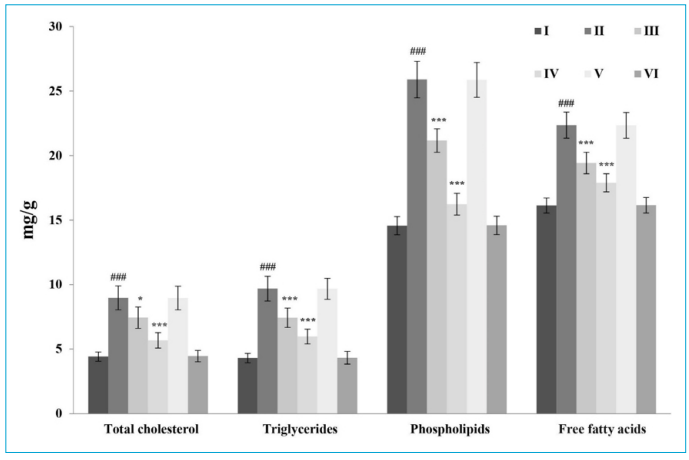

Figure 3. Effect of CAP and CAP@CS-NP on lipid profile in mammary tissue of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###p<0.001 when compared with control group and *p<0.05, ***p<0.001 when compared with DMBA group.

changes were found in CS-NP 5mg/kg b.wt (Group V) treated rats when compared to DMBA induced rats (Group II), and no significant differences were observed in Free CAP@NP (Group VI) alone treated rats when compared to control rats (Group I). Notably, CAP@CS-NP 4mg/kg b.wt was found to be more efficient than CAP 8mg/kg b.wt in modulating lipids levels.

Effect of CAP and CAP@CS-NP on Lipoprotein in Plasma

Figure 4 depicts the levels of lipoprotein cholesterol (HDL-C, LDL-C, and VLDL-C) in plasma of control and experimental rats. The levels of LDL-C and VLDL-C were significantly (p<0.001) raised up, whereas the levels of HDL-C were significantly (p<0.001) lowered in DMBA induced rats (Group II) when compared with the control rats (Group I). On the other hand, administration of CAP 8 mg/kg b.wt (Group III) and CAP@CS-NP 4mg/kg b.wt (Group IV) significantly depleted in LDL-C and VLDL-C and uplifted in HDL-C when compared with DMBA induced rats (Group II). No alterations were noted in CS-NP 5mg/kg b.wt (Group V) treated rats when compared with DMBA induced rats (Group II). However, the administration of Free CAP@NP (Group VI) alone treated rats did not cause any significant emendation when compared to control rats (Group I). Specifically, CAP@CS-NP 4mg/kg b.wt was found to be more powerful than CAP 8mg/kg b.wt in regulating lipoproteins levels.

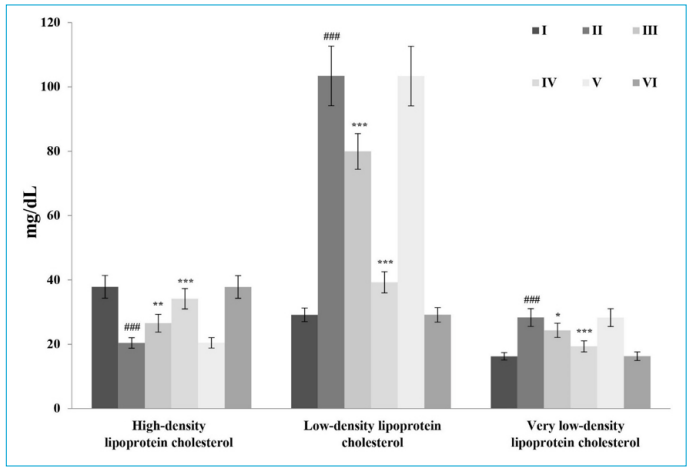

Figure 4. Effect of CAP and CAP@CS-NP on lipoproteins in plasma of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###p<0.001 when compared with control group and *p<0.05, **p<0.01, ***p<0.001 when compared with DMBA group

Effect of CAP and CAP@CS-NP on Glycoprotein Components in Plasma, Liver and Mammary Tissues

Figure 5, 6 and 7 displays the levels of glycoprotein components in plasma, liver, and mammary tissues of control and experimental rats. The levels of glycoprotein components namely hexose, hexosamine, and sialic acid were significantly (p<0.001) increased in DMBA induced rats (Group II) when compared with control rats (Group I). Contradictorily, administration of CAP 8mg/kg b.wt (Group III) and CAP@CS-NP 4mg/kg b.wt (Group IV) significantly decreased the levels of hexose, hexosamine, and sialic acid when compared with DMBA induced rats (Group II). No conversions were specified in CS-NP 5mg/kg b.wt (Group V) treated rats when compared with DMBA induced rats (Group II). Although, no significant differences were detected in Free CAP@NP (Group VI) alone treated rats when compared to

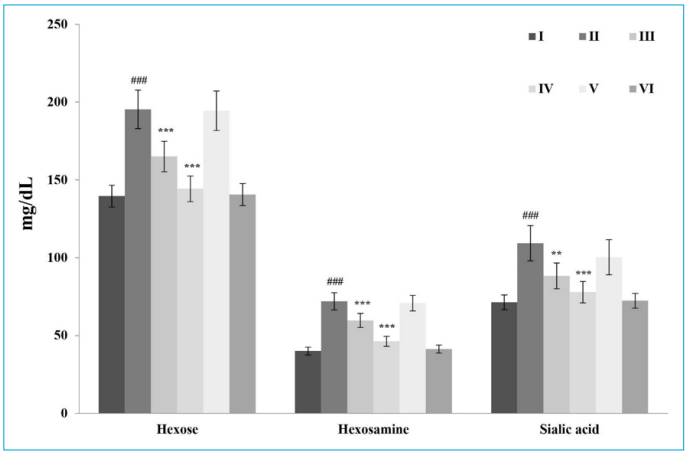

Figure 5. Effect of CAP and CAP@CS-NP on glycoprotein components in plasma of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###p<0.001 when compared with control group and **p<0.01, ***p<0.001 when compared with DMBA group.

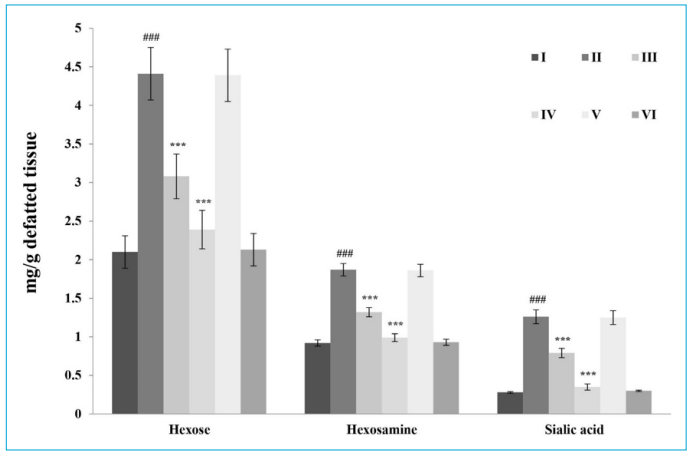

Figure 7. Effect of CAP and CAP@CS-NP on glycoprotein components in mammary tissue of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###p<0.001 when compared with control group and ***p<0.001 when compared with DMBA group.

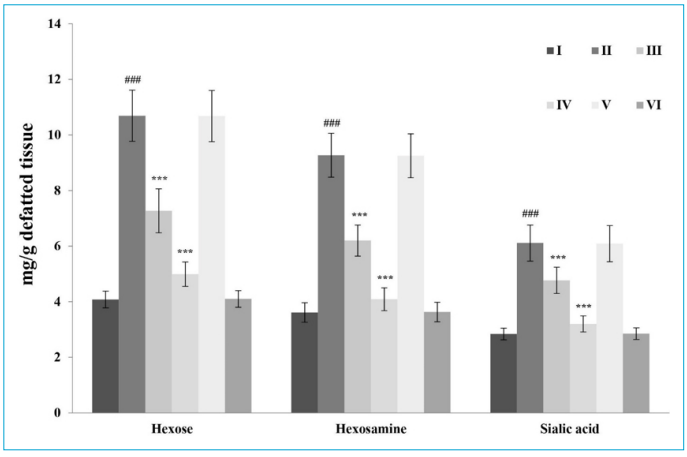

Figure 6. Effect of CAP and CAP@CS-NP on glycoprotein components in liver tissue of control and experimental rats. Values are expressed as mean±SD for six rats in each group. Significant levels are ###p<0.001 when compared with control group and ***p<0.001 when compared with DMBA group

control rats (Group I). Particularly, CAP@CS-NP 4mg/kg b.wt was found to be more impactful than CAP 8mg/kg b.wt in modifying glycoprotein components levels.

Effect of CAP and CAP@CS-NP on Histopathological Changes in Mammary Tissues

Figure 8 (A–F) illustrates the PAS staining analysis of glycoprotein in mammary tissues of control and experimental rats. The levels of glycoprotein were drastically raised in DMBA induced rats (Group II) (B) when compared with control rats (Group I) (A). On the other hand, administration of CAP 8mg/kg b.wt (Group III) (C) and CAP@CS-NP 4mg/kg b.wt (Group IV) (D) greatly constricted the levels of glyco-

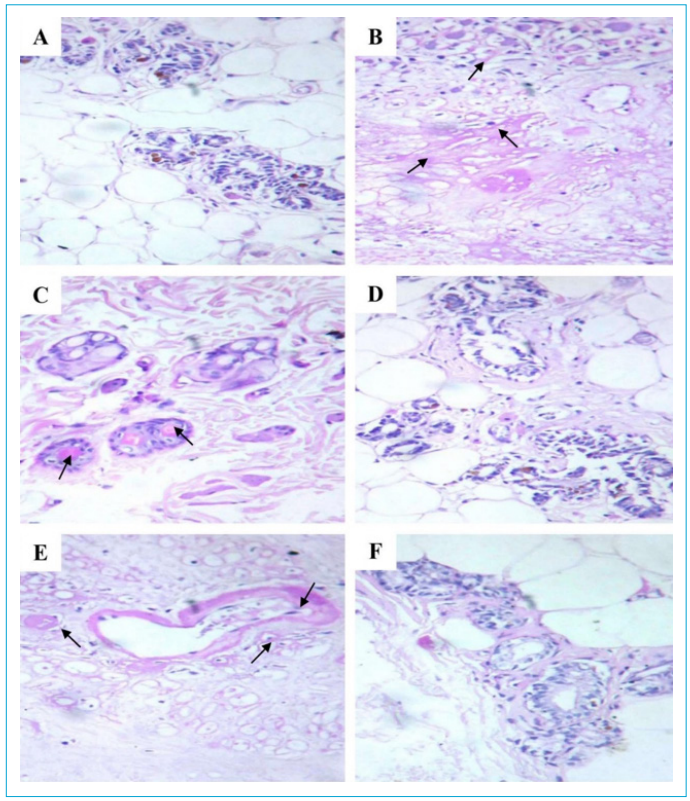

Figure 8. Effect of CAP and CAP@CS-NP on histopathological analysis of PAS staining in mammary tissue of control and experimental rats **(A-F)**. Control **(A)** and Free CAP@NP **(F)** alone treated rats showed normal mammary tissue staining. DMBA induced **(B)** and CS-NP 5mg/kg b.wt **(E)** treated rats showed increased levels of glycoprotein. CAP 8mg/kg b.wt **(C)** and CAP@CS-NP 4mg/kg b.wt **(D)** treated rats showed decreased levels of glycoprotein as compared to DMBA induced **(B)**.

protein when compared with DMBA induced rats (Group II) (B). No transformations were marked in CS-NP 5mg/kg b.wt (Group V) (E) treated rats when compared with DMBA induced rats (Group II) (B). However, no differences were spotted in Free CAP@NP (Group VI) (F) alone treated rats when compared to control rats (Group I) (A). Noteworthy, CAP@CS-NP 4mg/kg b.wt was shown to be more suitable than CAP 8mg/kg b.wt in alleviating glycoprotein accumulation.

Discussion

A vast majority of studies have evidenced the connection of lipids and lipoproteins with the peril of breast cancer.[33,34] The specific process by which lipids and lipoproteins promote cancer development is unclear. According to an earlier study, lipids may predominantly disrupt the gonads, with greater estradiol secretion influencing the progression of malignancies in the mammary glands and lymphoid system.[35] Triacylglycerides, phosphoglycerides, sterols, and sphingolipids are forms of lipids, a group of water-insoluble molecules. They play a variety of critical tasks at cellular and organism grades. Lipid metabolism abnormalities are becoming better recognized as an attribute of cancer cells. Semb et al.[36] have proposed that the increased lipid metabolism may be due to enhanced lipogenesis and defective degradation of lipids induced by tumor necrosis factor in cancer. In the present study, we heeded an increase in TC concentration in DMBA-induced rats, which could be owing to an upsurge in the concentration of LDL-C as it is the principal cholesterol transporter. However, CAP@CS-NP administration to tumor-bearing rats significantly altered abnormal levels to near normalcy.

TG is molecules that store and transfer the majority of the fat in a diet. Fatty acids are required for the formation of TG, which are used mostly for energy storage.[37] High TG levels are linked to a greater risk of major health problems. In this work, TG levels were identified to be significantly higher in DMBA induced tumor-bearing rats. Conversely, CAP@CS-NP administration significantly lowered the abnormal levels. Our study outcomes paralleled with the findings of Isabella and Mirunalini, in which they have proved the protective effect of 3, 3'-Diindolylmethane encapsulated CS nanoparticles in prop up with lipid metabolism against mammary cancer.[38] PL forms a special class of lipids which composed of a glycerol molecule substituted by one or two fatty acids and one additional polar group. DMBA-induced rats exhibit elevated levels of PL in their plasma, liver, and mammary tissues when compared with the control rats. Meanwhile, CAP@CS-NP administration was significantly reverted back to near the normal level. The finding of this study was also in harmony with the previous report.[38] FFA is usually expressed all over the body, but it is most abundant in the pancreas and gut, which has sparked interest as a potential target for diabetes and other metabolic illnesses. Prior researches have shown that elevated levels of FFA can stimulate the onset and progression of a wide variety of tumors.[39] Likewise, in the current work, DMBA induced cancer-bearing rats displayed an increased level of FFA in plasma, liver, and mammary tissues, which upon treatment with CAP@CS-NP significantly altered these levels to near-normal range. Endogenous lipids are rendered compatible with the aqueous environment of bodily fluids by lipoproteins, which are complex aggregates of lipids and proteins. HDL-C, LDL-C, and VLDL-C are the three types of lipoprotein cholesterol. Our finding shows that higher levels of LDL and VLDL and lower levels of HDL in DMBA induced tumor-bearing rats. Whereas, CAP@CS-NP were significantly reverted back to near-normal, which might be due to its powerful anti-lipidemic quality. Glycoproteins are essential constituents of cell membranes, play a crucial role in cell adhesion, intracellular processing of proteins, cell differentiation, signal transduction, host–pathogen interactions, cell activation, and capacity of cancer cells to metastasize.[40] Components of glycoproteins are hexose, hexosamine, and sialic acid. Increased amounts of these components in cancerous situations are valuable indications of the carcinogenic process, and these abnormalities affect the structure and function of cell membranes. In this study, we observed an increased level of hexose, hexosamine and sialic acid in DMBA induced tumor-bearing rats. Veena et al. also reported that elevated levels of glycoprotein components in plasma and tissues of cancer-bearing rats, which could be owing to the damage of connective tissues in the mammary tumor.[41] Treatment of CAP@CS-NP to tumor-bearing rats significantly inhibit the increasing levels of hexose, hexosamine, and sialic acid, this might be due to its anti-tumor and anti-metastatic properties.

PAS is a histological staining technique for detecting polysaccharides like glycoproteins, which is found on the surface of the lipid bilayer of cell membranes. The presence of elevated glycoprotein content in tumor tissue is indicated by increased PAS staining, which is accordance with the earlier reports of Arivazhagan and Sorimuthu Pillai.[42] Heavy intensity of PAS staining exhibits significantly greater in cancer conditions, which might imply that cancer cells are qualified to metastasis. In this investigation, DMBA induced tumor-bearing rats showed high accumulation of glycoprotein. However, CAP@CS-NP administration significantly decreased the glycoprotein levels when compared to tumor-bearing cancer rats. These outcomes strongly revealed the anti-tumor and anti-cancer activity of CAP@CS-NP.

Conclusion

From this study, it can be concluded that higher levels of lipids, lipoproteins, and glycoproteins could be a favorable prognostic factor for breast cancer. DMBA induces aberrant alterations in lipids, lipoproteins, and glycoproteins were successfully regulated by CAP@CS-NP, suggesting its therapeutic character against mammary carcinogenesis. Therefore, our result deserves further evaluation for use in human breast cancer cases.

Disclosures

Ethics Committee Approval: The study was approved by the Institutional Animal Ethics Committee (IAEC), regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (Reg No. 160/1999/CPCSEA and Proposal No. 1203).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – K.D.; Design – K.D., M.S.; Supervision – M.S.; Materials – K.D., M.V.; Data collection &/or processing – K.D., M.V.; Analysis and/or interpretation – K.D., M.S.; Literature search – M.V.; Writing – K.D.; Critical review – K.D., M.V., M.S.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin 2020;71:209–49.
2. Nandakumar N, Rengarajan T, Balamurugan A, Balasubramanian MP. Modulating effects of hesperidin on key carbohydrate-metabolizing enzymes, lipid profile, and membrane-bound adenosine triphosphatases against 7, 12-dimethylbenz (a) anthracene-induced breast carcinogenesis. Hum Exp Toxicol 2014;33:504–16.
3. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J 2012;279:2610–23.
4. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature 2012;491:364–73.
5. Oliveira-Ferrer L, Legler K, Milde-Langosch K. Role of protein glycosylation in cancer metastasis. Semin Cancer Biol 2017;44:141–52.
6. Periyasamy K, Baskaran K, Ilakkia A, Vanitha K, Selvaraj S, Sakthisekaran D. Antitumor efficacy of tangeretin by targeting the oxidative stress mediated on 7, 12-dimethylbenz (a) anthracene-induced proliferative breast cancer in Sprague–Dawley rats. Cancer Chemother Pharmacol 2015;75:263–72.
7. Hamzehzadeh L, Atkin SL, Majeed M, Butler AE, Sahebkar A. The versatile role of curcumin in cancer prevention and treatment: A focus on PI3K/AKT pathway. J Cell Physiol 2018;233:6530–7.
8. Khorsandi K, Kianmehr Z, Hosseinzadeh R. Anti-cancer effect of gallic acid in presence of low level laser irradiation: ROS production and induction of apoptosis and ferroptosis. Cancer Cell Int 2020;20:18.
9. Chapa-Oliver AM, Mejia-Teniente L. Capsaicin: From plants to a cancer-suppressing agent. Molecules 2016;21:931–9.
10. Park SY, Kim JY, Lee SM, Jun CH, Cho SB, Park CH, et al. Capsaicin induces apoptosis and modulates MAPK signaling in human gastric cancer cells. Mol Med Rep 2014;9:499–502.
11. Friedman JR, Perry HE, Brown KC, Gao Y, Lin J, Stevenson CD, et al. Capsaicin synergizes with camptothecin to induce increased apoptosis in human small cell lung cancers via the calpain pathway. Biochem Pharmacol 2017;129:54–66.
12. Zhu M, Yu X, Zheng Z, Huang J, Yang X, Shi H. Capsaicin suppressed activity of prostate cancer stem cells by inhibition of Wnt/β-catenin pathway. Phytother Res 2020;34:817–24.
13. Kalaiyarasi D, Mirunalini S. Capsaicin (Capsicum Annuum): A ubiquitous compound with multivarient pharmaceutical properties. Res J Chem Environ 2021;25:234–40.
14. Singh T, Shukla S, Kumar P, Wahla V, Bajpai VK, Rather IA. Application of nanotechnology in food science: perception and overview. Front Microbiol 2017;8:1–7.
15. Holm BA, Bergey EJ, De T, Rodman DJ, Kapoor R, Levy L, et al. Nanotechnology in biomedical applications. Mol Cryst Liq 2002;374:589–98.
16. Kalaiyarasi D, Manobharathi V, Mirunalini S. Development of nano drugs: A promising avenue for cancer treatment. Res J Biotechnol 2021;16:234–44.
17. Das RK, Kasoju N, Bora U. Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomed-Nanotechnol 2010;6:153–60.
18. Abd Elgadir M, Uddin MS, Ferdosh S, Adam A, Chowdhury AJ, Sarker MZ. Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: A review. J Food Drug Anal 2015;23:619–29.
19. Arulmozhi V, Pandian K, Mirunalini S. Ellagic acid encapsulated chitosan nanoparticles for drug delivery system in human oral cancer cell line (KB). Colloids Surf B 2013;110:313–20.
20. Chidambaram, N, Baradarajan, A. Influence of selenium on glutathione and some associated enzymes in rats with mammary tumor induced by 7, 12-dimethylbenz (a) anthracene. Mol Cell Biochem 1996;156:101–7.
21. Anandakumar P, Kamaraj S, Jagan S, Ramakrishnan G, Asokkumar S, Naveenkumar C, et al. The anti-cancer role of capsaicin in experimentally induced lung carcinogenesis. J Pharmaco punct 2015;18:19–25.
22. Folch J, Lees M, Stanley GS. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509.
23. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med 1953;41:486–

92.
24. Foster LB, Dunn RT. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin Chem 1973;19:338–40.
25. Zilversmit DB, Davis AK. Micro determination of plasma phospholipids by trichloroacetic acid precipitation. J Lab Clin Med 1950;35:155–60.
26. Falholt K, Lund B, Falholt W. An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clin Chim Acta 1973;46:105–11.
27. Wilson DE, Spiger MJ. A dual precipitation method for quantitative plasma lipoprotein measurement without ultracentrifugation. J Lab Clin Med 1973;82:473–82.
28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
29. Niebes P. Determination of enzymes and degradation products of glycosaminoglycan metabolism in the serum of healthy and varicose subjects. Clin Chim Acta 1972;42:399–408.
30. Elson LA, Morgan WT. A colorimetric method for the determination of glucosamine and chondrosamine. Biochem J 1933;27:1824–8.
31. Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem 1959;234:1971–5.
32. Yamabayashi S. Periodic acid-Schiff-Alcian Blue: A method for the differential staining of glycoproteins. Histochem 1987;19:565–71.
33. Baumann J, Sevinsky C, Conklin DS. Lipid biology of breast cancer. BBA-Mol cell biol 2013;1831:1509–17.
34. Ray G, Husain SA. Role of lipids, lipoproteins and vitamins in women with breast cancer. Clin Biochem 2001;34:71–6.
35. Pattanayak SP, Sunita P, Mazumder PM. Restorative effect of Dendrophthoe falcata (Lf) Ettingsh on lipids, lipoproteins, and lipid-metabolizing enzymes in DMBA-induced mammary gland carcinogenesis in Wistar female rats. Comp Clin Path 2014;23:1013–22.
36. Semb H, Peterson J, Tavernier J, Olivecrona T. Multiple effects of tumor necrosis factor on lipoprotein lipase in vivo. J Biol Chem 1987;262:8390–4.
37. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J 2012;279:2610–23.
38. Isabella S, Mirunalini S. Protective effect of 3, 3′ -Diindolylmethane encapsulated chitosan nanoparticles prop up with lipid metabolism and biotransformation enzymes against possible mammary cancer. J Appl Pharm Sci 2017;7:194–201.
39. Wu Y, Yu X, Yi X, Wu K, Dwabe S, Atefi M, et al. Aberrant phosphorylation of SMAD4 Thr277-mediated USP9x–SMAD4 interaction by free fatty acids promotes breast cancer metastasis. Cancer Res 2017;77:1383–94.
40. Wang Y, Ao X, Vuong H, Konanur M, Miller FR, Goodison S, et al. Membrane glycoproteins associated with breast tumor cell progression identified by a lectin affinity approach. J Proteome Res 2008;7:4313–25.
41. Veena K, Shanthi P, Sachdanandam P. Anti-cancer effect of Kalpaamruthaa on mammary carcinoma in rats with reference to glycoprotein components, lysosomal and marker enzymes. Biol Pharm Bull 2006;29:565–9.
42. Arivazhagan L, Pillai SS. Tangeretin, a citrus pentamethoxyflavone, exerts cytostatic effect via p53/p21 up-regulation and suppresses metastasis in 7, 12-dimethylbenz (α) anthracene-induced rat mammary carcinoma. J Nutr Biochem 2014;25:1140–53.