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. 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
influence on the neoplastic process, including alteration in the lipid metabolism and glycoprotein components status. 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. 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. 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.

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. 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. 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. 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. 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 physicochemical qualities. CS formulations with a high level of deacetylation are recommended in drug delivery systems because of their greater degradation rate.

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.

**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. 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. 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. TC in plasma, liver, and mammary tissues were estimated by using the kit method of Zlatkis et al. TG in plasma, liver, and mammary tissues were measured by the method of Foster and Dunn. PL in plasma, liver, and mammary tissues were determined by the method of Zilversmit and Davis. FFA in plasma, liver, and mammary tissues were estimated by the method of Falholt et al. HDL-C in plasma was estimated by the method of Wilson and Spiger using a reagent kit. LDL-C and VLDL-C in plasma were calculated by Friedwald et al. LDL-C = TC – (HDL-C + VLDL-C), VLDL-C= TG/5.

**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.

**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<0.01, ##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.

**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). Contradictory, 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
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-
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. 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. Triacylglycerides, phosphoglycerides, sterols, and of malignancies in the mammary glands and lymphoid system with greater estradiol secretion influencing the progression of malignancies in the mammary glands and lymphoid system.

Meanwhile, CAP@CS-NP 4mg/kg b.wt was significantly reverted back to near the normal level. The finding of this study was also in harmony with the previous report. 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. 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.

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. Vee-una 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. 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. 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).

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**Conflict of Interest:** None declared.


**References**


