Chemotherapeutic effect of 3, 3′-Diindolylmethane encapsulated chitosan nanoparticles on 7, 12-Dimethylbenz (a) anthracene induced mammary cancer – A dose dependent study

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**A R T I C L E   I N F O**

Article history:
Received 1 February 2016
Received in revised form 3 April 2016
Accepted 22 April 2016
Available online 26 April 2016

**Keywords:**
Breast cancer
DIM
DMBA
Antioxidant
Oxidative stress
Nanoencapsulation

**A B S T R A C T**

Breast cancer is the second most prevalent cancer among women and its incidence is amplifying alarmingly. Since genetic factors are believed to account for only 10% of the reported cases, remaining the environmental factors, including diet are thought to play a significant role in predisposing breast cancer. Many bioactive compounds have been reported for their anticancer potential. One among the bioactive compound 3, 3′-Diindolylmethane (DIM) is a phytochemical possess a wide array of pharmacological activities such as anti-proliferative and anti-oxidant properties. Its properties such as poor water solubility and low bioavailability have hampered its clinical development. Therefore, it is a great interest to study whether the nano formulation for DIM with chitosan for its enhanced potential, the present study was aimed to evaluate the chemotherapeutic potential of 3, 3′-Diindolylmethane encapsulated chitosan nanoparticles (DIM@CS-NP) on 7,12-Dimethylbenz(a)anthracene (DMBA) induced mammary carcinoma in rats. DMBA was induced in a single subcutaneous injection of 25 mg/ kg body weight to each rat. In the present study, we investigated the altered activities of lipid peroxidation, enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidant (GSH) in plasma, liver and mammary tissue, supported by histopathological study of mammary tissues. We evaluated the changes in the body weight of control and experimental animals. There was a significant decrease in the final body weight of tumor bearing animals, when compared to control animals. Also, we observed a diminished cellular antioxidant status and the elevated oxidant levels in plasma, liver, mammary tissues of DMBA induced rats. However, administration of DIM@CS-NP significantly increased the mean final body weight when compared with DMBA induced animals. Oral supplementation of different doses of DIM@CS-NP (0.5, 1, 2 mg/kg BW), significantly renovated the activities of cellular antioxidants and ultimately diminished the levels of lipid peroxidation, which pointed towards suppression of preneoplastic lesions, thereby reduced the cancer risk, and significant improvement in the levels of enzymatic (SOD, CAT, GPx) and non-enzymatic antioxidant (GSH) in the plasma, liver and mammary tissue. Based on the above finding we conclude the nano formulation of DIM provides a novel therapeutic regime for mammary cancer.

**Focal points:**

- Benchside
  - Oxidants, antioxidant status and histopathological examination of mammary tumor tissue are necessary to determine the chemotherapeutic potential of DIM@CS-NP on experimental induced rat mammary carcinoma.
- Bedside
  - Ingestion of DIM@CS-NP may reduce the severity of DMBA induced tumor bearing animals. More over nanoformulated drug delivery systems from biocompatible and biodegradable polymers contributes an evolving approach in drug delivery and tumor targeting.
1. Introduction

Breast cancer is the second major widespread cancer in women worldwide, accounts for the highest morbidity and mortality [1]. In addition, 23% of the entire cancer cases and 14% of the cancer death are due to the breast cancer. The prevalence of breast cancer was reported in 151 countries worldwide [2]. The etiology of breast cancer is multifactorial and the jeopardy factors consist of early on menarche, delayed menopause, nulliparity, post-menopausal obesity, hormone substitution therapy, comprehensive makes use of an oral contraceptives, family history and previous benign breast disease [3]. One in eight women is affected with breast cancer throughout their life span [4].

Precisely oxidative stress plays an imperative task in the pathogenesis of cancer. When there is production of reactive oxygen species (ROS), caused by the ecological factors like UV radiation, ionizing radiation, xenobiotics, tobacco and smoke as well as in activation of superficial receptors. The production of reactive oxygen species (ROS) is larger than the body's ability to detoxify the reactive intermediates. The imbalance between ROS and antioxidant defense mechanisms leads to life-threatening diseases. Thereby, frequently producing free radicals/non-radical and oxidizing species all along with electrophiles mediates, oxidative stress favoring damage of nucleic acid, proteins and lipids. In these circumstances, chromosomal instability, mutation, loss of organelle functions, membrane damage leading to, development of carcinogenesis [5]. In this scenario, antioxidants act as free radical scavengers, slow down lipid peroxidation (LPO) and other free radical-mediated procedure thereby defending the human body from oxidative stress favoring damage of nucleic acid, proteins and lipids. Indeed, free radical scavengers like SOD, CAT, and GPx are inhibited at different stages of carcinogenesis [6]. Conventionally, the industrialization of contemporary society generates a variety of environmental pollutants that eventually contribute to breast cancer [8]. Polycyclic aromatic hydrocarbon is one of the major environmental contaminants known for capacity to generate free radicals that induce oxidative stress, DMBA a well known typical polycyclic aromatic hydrocarbon (PAH), binds with DNA responsible for mutagenicity and carcinogenicity [9]. Furthermore, several epidemiologic studies exposed that an association connecting PAH-DNA adducts and breast cancer incidence [10,11]. Animal experimental systems are predominantly in use for the study of human mammary carcinogenesis. Since the rat mammary gland demonstrates a high susceptibility to budding neoplasms which closely mimic human breast cancer, they have been selected for the comparison to other animal replica [12].

Many bioactive compounds of plant origin have the possible to collapse the biochemical imbalances induced by the development of free radicals. For this reason, naturally occurring antioxidants have been analyzed as promising chemotherapeutic drugs for the management of cancer without causing damage to normal cells [13]. One such dietary bioactive compound 3, 3'-Diindolylmethane (DIM) is a phytochemical naturally produced during the autolytic breakdown of glucobrassicin. It is phytochemical is highly found in the food plants of Brassica genus such as Brussels sprouts, cauliflower, cabbage, kale, horseradish and wasabi. Fig. 1 shows the structure of DIM. DIG is a potential anti-tumor mediator that has been widely studied in laboratory and clinical trials [14,15]. Recent studies confirmed that DIG protected the cell against reactive oxygen species (ROS) in a breast cancer susceptibility gene 1 (BRCA1) needing manner, [16] also induce apoptosis of breast cancer cells [17]. Many studies are finished with DIG in a range of cell lines, especially DIG induced a G1 cell cycle arrest in human breast cancer MCF-7 cells by a mechanism that induced increased expression of p21, followed by, DIG treatment induced hyper polarization of mitochondrial inner membrane, decreased cellular ATP level, and significantly stimulated mitochondrial reactive oxygen species (ROS) production [18]. Despite its well reputable scientific records as an anticancer agent in copious preclinical models, its properties such as poor water solubility and low bioavailability are the most important roadblocks in its development as an anticancer agent [19]. Nanoscale drug delivery systems formulated from biocompatible and biodegradable polymers contribute a developing approach to drug delivery and tumor targeting. Biodegradable drug carrier are being engineered and constructed with nanometer dimensions [20]. Yang Chao Luo et al. also synthesis the encapsulation of hydrophobic bioactive in Zein/CMCS nanoparticles is a promising approach to get better their stability adjacent to harsh condition and provide controlled release of food/pharmaceutical applications [21]. Such approaches made it possible to develop a great interest in investigating the encapsulation of DIG in nanoparticles could provide a sustained release property and enhance their stabilities and bioavailability from harsh environmental conditions. Indeed, the use of controlled drug release systems has certain advantages compared to conventional forms of dosages, as they can diminish side effects, and also extend the efficacy of the drug.

![Fig. 1. Structure of 3, 3'-Diindolylmethane.](image-url)
Undeniably, numerous carriers are involved for an effective drug delivery system such as liposome, polymeric nanoparticles, biodegradable microsphere, cyclodextrin and hydrogel [22]. Chitosan (CS) is one among the promising nano-carrier to liberate the encapsulated anticancer drug to the tumor tissues [23]. Moreover, drug delivery systems based on Chitosan are interesting with regard to regulate the drug release to the physiological requirements of the body, as in the case of hormone release [24]. In the present study, we designed with an objective to investigate the 3, 3′-Diindolylmethane encapsulated Chitosan nanoparticles [DIM@CS-NP] on DMBA induced Sprague Dawley rats by evaluating oxidant, antioxidant and histopathological changes.

2. Materials and methods

2.1. Chemicals and reagent

3, 3′-Diindolylmethane (DIM), chitosan, 7,12-dimethylbenz(a) anthracene (DMBA) were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, thiobarbituric acid (TBA), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT) and reduced glutathione (GSH), dimethyl sulphoxide (DMSO) were purchased from Himedia. All the other chemicals used in this study were of analytical grade available commercially.

2.2. Animal model

Female Sprague Dawley rats six to ten weeks old, weighed 130–150 g was purchased from National Institute of Nutrition, Hyderabad, India. After obtaining approval from the Institutional Animal Ethics Committee for the Control and direction of Experimental Animal (CPCSEA approval no: 1123) guidelines. Feed and water provided. Animals were maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamilnadu, India.

2.3. Induction of mammary carcinogenesis

Mammary cancer was induced in Sprague Dawley rat using a single subcutaneous injection of 25 mg/kg BW of DMBA in 1 mL emulsion of sunflower oil (0.75 mL) and physiological saline (0.25 mL) to each rat beneath the mammary gland on either side [25].

2.4. Experimental design

Dose fixation study, rats were divided into ten groups and each group consisting six animals. Group I rats were served as untreated control and provided animal food pellet and water throughout the experimental period. Groups II to VIII rats were carried single doses of 25 mg/kg BW of DMBA, in 0.75 mL of sunflower oil and 0.25 mL of physiological saline as a single subcutaneous injection during the first week of the experiment. Group II rats, no other treatment. After 60 days the cancer-bearing groups III-V rats administered orally DIM different doses (5, 10, 20 mg/kg BW) and groups VI - VIII were received orally DIM@CS-NP different doses (0.5, 1, 2 mg/kg BW) respectively for every alternative days. Finally, rats in groups of IX & X where received DIM alone (20 mg/kg BW) in 0.5% of DMSO and DIM @ CS-NP (2 mg/kg BW) both were served as drug control.

2.5. Collection of blood and tissues

At the end of the 16th week, all the rats were fasted overnight and sacrificed by cervical decapitation. Blood samples were collected in heparinised tubes and the plasma was used for the biochemical analysis. Liver and mammary tissues were excised from the rats and stored in ice-cold containers immediately. Tissues were homogenized with appropriate buffer, centrifuged at 3000 g and the supernatant were used for biochemical evaluation on the same day of sacrifice. Liver and mammary tissues also potted in 10% formalin and stored at –8 °C for histopathological studies.

2.6. Histopathological examination

The histopathology analysis of mammary tissues in control and experimental animals was sliced and immersed at once in 10% buffered formalin solution for fixation and dehydrated with graded ethanol solutions from 50% to 100% and then embedded in paraffin. Sections of 5 μm in thickness were cut and stained with haematoxylin and eosin and the slides were observed under the microscope.

2.7. Biochemical analysis

2.7.1. Estimation of lipid peroxidation

The levels of thiobarbituric acid reactive substances (TBARS) in plasma were estimated by the method of Yagi [26]. In mammary and liver tissues were investigated by the method of Ohkawa et al., [27]. SOD status was estimated by the method of Kakkar et al., [28]. CAT activities were examined by the method of Sinha [29]. GPx activities were determined by the method of Rotruck et al. [30]. GSH levels were determined by the method of Ellman [31].

2.8. Statistical analysis

Statistical analyses were carried out using SPSS 17 (SPSS, Inc., Chicago) statistical package. The statistical data were shown as mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) comparison method was used to associate the difference between the variables. The Data were expressed significant if p values are less than 0.05.

3. Results

3.1. Body weight changes

Table 1 showed the changes in the body weight of control and experimental animals. Initially, there were no significant changes in the body weight of control and experimental animals. At the end of the experiment, we observed significantly (p < 0.05) diminished in the body weight of DMBA-induced tumor bearing animals (group II) when compared to control group. However, administration of DIM and DIM@CS-NP significantly (p < 0.05) increased the mean final body weight when compared with group II animals. No significant changes were observed in control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight</th>
<th>Final body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>134.10 ± 12.43a</td>
<td>161.20 ± 11.86a</td>
</tr>
<tr>
<td>DMBA</td>
<td>142.50 ± 13.65a</td>
<td>96.22 ± 8.74a</td>
</tr>
<tr>
<td>DMBA + DIM 5 mg/kg BW</td>
<td>145.00 ± 12.93a</td>
<td>106.39 ± 10.66a</td>
</tr>
<tr>
<td>DMBA + DIM 10 mg/kg BW</td>
<td>143.50 ± 13.16a</td>
<td>132.71 ± 6.49a</td>
</tr>
<tr>
<td>DMBA + DIM 20 mg/kg BW</td>
<td>144.50 ± 13.65a</td>
<td>139.88 ± 10.11a</td>
</tr>
<tr>
<td>DMBA DIM@CS-NP 0.5 mg/kg BW</td>
<td>142.50 ± 11.20a</td>
<td>155.60 ± 11.78a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 1 mg/kg BW</td>
<td>141.50 ± 14.01a</td>
<td>155.68 ± 12.28a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 2 mg/kg BW</td>
<td>142.00 ± 12.39a</td>
<td>155.86 ± 12.92a</td>
</tr>
<tr>
<td>DIM 20 mg/kg BW</td>
<td>138.10 ± 12.33a</td>
<td>163.60 ± 11.9a</td>
</tr>
<tr>
<td>DIM@CS-NP 2 mg/kg BW</td>
<td>139.00 ± 11.69a</td>
<td>170.09 ± 14.29a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Values not share a common superscripts differ significantly at a p – value of < 0.05 (DMRT).
control treated groups. However DMBA treated with DIM@CS-NP rats revealed increased body weight near control when compared to DMBA and DIM.

Table 2 Effect of DIM and DIM@CS-NP on tumor incidence and tumor volume in control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Animals with tumor</th>
<th>Tumor incidence (%)</th>
<th>Tumor volume (mm³)/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DMBA</td>
<td>6/6</td>
<td>100</td>
<td>23.20 ± 2.32*</td>
</tr>
<tr>
<td>DMBA + DIM 5 mg/kg BW</td>
<td>4/6</td>
<td>66</td>
<td>14.49 ± 1.21*</td>
</tr>
<tr>
<td>DMBA + DIM 10 mg/kg BW</td>
<td>2/6</td>
<td>33</td>
<td>11.80 ± 0.98e</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 0.5 mg/kg BW</td>
<td>2/6</td>
<td>33</td>
<td>11.71 ± 0.89f</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 1 mg/kg BW</td>
<td>1/6</td>
<td>16.7</td>
<td>5.63 ± 0.44d</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 2 mg/kg BW</td>
<td>0/6</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>DIM@CS-NP 2 mg/kg BW</td>
<td>0/6</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Values not share a common superscripts differ significantly at a p value of < 0.05 (DMRT).

Table 3 Effect of free DIM and DIM@CS-NP on plasma TBARS and antioxidant status in control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (mM/dL)</th>
<th>SOD (U*/mL)</th>
<th>CAT (U**/mL)</th>
<th>GPX (U***/mL)</th>
<th>GSH (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.89 ± 0.19a</td>
<td>4.66 ± 0.35a</td>
<td>2.67 ± 0.17a</td>
<td>93.27 ± 7.85a</td>
<td>36.59 ± 2.51a</td>
</tr>
<tr>
<td>DMBA</td>
<td>5.67 ± 0.25b</td>
<td>2.76 ± 0.13b</td>
<td>1.26 ± 0.11b</td>
<td>61.24 ± 4.76b</td>
<td>22.00 ± 1.76b</td>
</tr>
<tr>
<td>DMBA + DIM 5 mg/kg BW</td>
<td>4.82 ± 0.23c</td>
<td>3.63 ± 0.22c</td>
<td>1.82 ± 0.15c</td>
<td>81.77 ± 6.75a</td>
<td>28.78 ± 2.44a</td>
</tr>
<tr>
<td>DMBA + DIM 10 mg/kg BW</td>
<td>4.10 ± 0.24d</td>
<td>4.12 ± 0.25d</td>
<td>2.20 ± 0.13d</td>
<td>90.89 ± 6.31a</td>
<td>34.08 ± 2.99a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 0.5 mg/kg BW</td>
<td>4.06 ± 0.22e</td>
<td>4.14 ± 0.30e</td>
<td>2.23 ± 0.12e</td>
<td>90.91 ± 8.97a</td>
<td>34.12 ± 3.21a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 1 mg/kg BW</td>
<td>3.14 ± 0.28f</td>
<td>4.45 ± 0.31f</td>
<td>2.58 ± 0.20f</td>
<td>92.65 ± 7.22a</td>
<td>35.63 ± 3.43a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 2 mg/kg BW</td>
<td>3.11 ± 0.27g</td>
<td>4.48 ± 0.33g</td>
<td>2.61 ± 0.19g</td>
<td>92.31 ± 7.53a</td>
<td>35.65 ± 2.88a</td>
</tr>
<tr>
<td>DIM@CS-NP 2 mg/kg BW</td>
<td>3.09 ± 0.18h</td>
<td>4.51 ± 0.39h</td>
<td>2.62 ± 0.22h</td>
<td>92.34 ± 7.97a</td>
<td>35.68 ± 3.10a</td>
</tr>
<tr>
<td>DIM 20 mg/kg BW</td>
<td>2.92 ± 0.20i</td>
<td>4.62 ± 0.32i</td>
<td>2.65 ± 0.17i</td>
<td>93.13 ± 8.97a</td>
<td>36.50 ± 3.32a</td>
</tr>
<tr>
<td>DIM@CS-NP 2 mg/kg BW</td>
<td>2.90 ± 0.21j</td>
<td>4.65 ± 0.40j</td>
<td>2.66 ± 0.18j</td>
<td>93.20 ± 9.19a</td>
<td>36.52 ± 2.79a</td>
</tr>
</tbody>
</table>

U*: Amount of enzyme to inhibit 50% NBT reduction/min
U**: μmol of H₂O₂ consumed/min.
U***: μg of GSH consumed/min.

3.2. Effect of DIM@CS-NP on total number of tumors, tumor incidence and tumor volume in control and experimental animals

Table 2 showed the total number of tumors, tumor incidence and tumor volume of control and experimental animals. The study confirmed the development of tumor incidence to 100% in DMBA treated rats. The tumor weights were found to be significantly (p < 0.05) increased in Group II cancer-bearing animals when compared with control animals. Administration of DIM decreased the tumor weight in groups III, IV, V animals remarkably reduced the tumor incidence as 66%, 33% and 33% respectively, when compared to Group II animals. Further, in VI group DIM@CS-NP 0.5 mg/kg BW treated animals, there was a significantly (p < 0.05) decreased in tumor weights were found to be 16.7%. On the other hand, the administration of DIM@CS-NP 1 and 2 mg/kg BW there was significantly (p < 0.05) decreases in tumor weights were found equivalent effective as 16.7% correspondingly. There was, no tumor observed in Group IX and X (free DIM and DIM@CS-NP) alone treated animals when compared with Group I control animals.

3.3. Effect of free DIM and DIM@CS-NP on lipid peroxidation and antioxidant status in control and experimental animals

Tables 3, 4 and 5 showed the activities of lipid peroxidation (TBARS), enzymatic antioxidants (SOD, CAT, GPX) and non-enzymatic antioxidants (GSH) in liver, plasma and mammary tissue of control and experimental animals, respectively. The activities of TBARS were significantly (p < 0.05) increased, whereas the activities of SOD, CAT, GPX and GSH were significantly (p < 0.05)

Table 4 Effect of free DIM and DIM@CS-NP on liver TBARS and antioxidant status in control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (mM/100 g wet tissue)</th>
<th>SOD (U*/mg ptn)</th>
<th>CAT (U**/mg ptn)</th>
<th>GPX (U***/mg ptn)</th>
<th>GSH (U/mg ptn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.65 ± 0.05a</td>
<td>8.98 ± 0.75a</td>
<td>50.63 ± 4.10a</td>
<td>4.91 ± 0.42a</td>
<td>8.65 ± 0.62a</td>
</tr>
<tr>
<td>DMBA</td>
<td>2.30 ± 0.51b</td>
<td>4.49 ± 0.37b</td>
<td>28.41 ± 1.88b</td>
<td>2.18 ± 0.19b</td>
<td>5.43 ± 0.45b</td>
</tr>
<tr>
<td>DMBA + DIM 5 mg/kg BW</td>
<td>1.78 ± 0.27c</td>
<td>4.54 ± 0.25c</td>
<td>40.31 ± 3.21a</td>
<td>5.02 ± 0.29a</td>
<td>6.25 ± 0.42a</td>
</tr>
<tr>
<td>DMBA + DIM 10 mg/kg BW</td>
<td>0.63 ± 0.06d</td>
<td>6.04 ± 0.45d</td>
<td>47.41 ± 3.21a</td>
<td>3.18 ± 0.28a</td>
<td>7.22 ± 0.74d</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 0.5 mg/kg BW</td>
<td>0.81 ± 0.07e</td>
<td>6.32 ± 0.50e</td>
<td>47.49 ± 3.54a</td>
<td>3.23 ± 0.27a</td>
<td>7.27 ± 0.52d</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 1 mg/kg BW</td>
<td>0.74 ± 0.07f</td>
<td>7.90 ± 0.65f</td>
<td>49.03 ± 4.10a</td>
<td>4.27 ± 0.24d</td>
<td>8.23 ± 0.73a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 2 mg/kg BW</td>
<td>0.72 ± 0.06g</td>
<td>7.94 ± 0.65g</td>
<td>49.12 ± 4.54a</td>
<td>4.33 ± 0.23d</td>
<td>8.27 ± 0.84a</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 2 mg/kg BW</td>
<td>0.68 ± 0.06h</td>
<td>7.97 ± 0.66h</td>
<td>49.16 ± 4.43a</td>
<td>4.42 ± 0.37h</td>
<td>8.31 ± 0.63a</td>
</tr>
</tbody>
</table>

U*: Amount of enzyme to inhibit 50% NBT reduction/min.
U**: μmol of H₂O₂ consumed/min.
U***: μg of GSH consumed/min.

Values are expressed as mean ± SD for six animals in each group. Values not share a common superscripts differ significantly at a p value of < 0.05 (DMRT).
In the field of better therapeutic approach by increasing the site specificity, drug delivery and sustained released. Recently, Arulmozhi et al. [37] also reported that ellagic acid encapsulated chitosan nanoparticles for drug delivery system on hamster buccal pouch carcinogenesis, for those end chitosan is suitable biopolymer to use target drug delivery system.

DMBA is an effective laboratory carcinogen known to produce toxic and highly diffusible ROS, which are capable of producing deleterious effects [38]. In the present study, we reported a significant decrease in the body weight in DMBA treated rats, is because of nutritional diminution, expenditure of the host causing body weight loss may parallel a decrease in tumor volume [39]. In the present analysis, the food and water intake in different investigations groups were found to be unaltered. The total body weights of the cancer-induced group II animals were declined when compared to control groups. This may be due to the energy modify metabolism during tumor formation and in addition an increased level of lipid peroxidation plays an imperative role in the initiation of tumor development and in the reduction of body weight [40]. Increased body weight was observed in DIM@CS-NP treated groups compared to group II. This clearly indicates that DIM@CS-NP inhibited LPO as extended level due to the antioxidant activity of DIM@CS-NP.

There is a dynamic balance linking between the amount of free radicals produced in the body and antioxidant defense system that extinguish or scavenge them and protect their body against the harmful effects. Antioxidants may defend against the toxicity of reactive oxygen species (ROS) by the impediment of ROS formation [41]. The analysis of antioxidant levels is measured essential as they neutralize oxygen-free radicals [42]. Life threatening disease development is linked to the availability of these antioxidants [43]. A previous study showed that the augmented level of TBARS in tumor cells is associated through overproduction of free radicals and provide an index to assess the size of tissue damage [44]. In the current study, we observed increased levels of TBARS in DMBA-induced animals. On the other hand DIM@CS-NP administration changed the lipid peroxidation status significantly indicating its anti-lipid peroxidative property.

Previous study suggests that the antioxidant enzymes are critical in protecting against tumor-promoting agents. Interestingly, malignant cells or transformation is often accompanied by diminishing in activity of antioxidant enzymes (SOD, CAT and GPx) which makes the cell disturbances to carcinogen [45]. In the study, we observed that decreased activities of enzymatic antioxidants such as SOD, CAT, GPx and non-enzymatic antioxidant GSH in DMBA alone treated animals. Administration of DIM@CS-NP to DMBA treated animals improves the status of antioxidant in a dose

decreased in DMBA alone treated animals when compared with the control group. Oral administration of DIM@CS-NP at different doses (0.5, 1, 2 mg/kg BW) and free DIM (5, 10, 20 mg/kg BW) were significantly (p < 0.05) decreased in TBARS, whereas significantly (p < 0.05) increased in the activities of SOD, CAT, GPx and GSH when compared with group II animals. However, there was no significantly (p < 0.05) difference in the animals administered with DIM@CS-NP alone when compared to control animals.

### 3.4. Histopathological changes in the mammary tissues of control and experimental animals (haematoxylin and eosin staining)

Fig. 2 (A–J) shows the histopathological changes in mammary tissues of control rats were showed (A), DMBA 20 mg/kg BW (I) and DIM@CS-NP 2 mg/kg BW (J) rats showing normal ductal epithelial architecture of the terminal epithelial buds. In contrast, mammary tissues of DMBA (25 mg/kg BW) induced rat (B) showed invasive ductal carcinoma with abnormal cellular proliferation and infiltrated ducts. Followed by mammary tissue of tumor bearing rats treated with DMBA 5 mg/kg BW (C) showed non invasive ductal carcinoma and DIM 10 & 20 mg/kg BW (C & D) moderate ductal hyperplasia respectively. Mammary tissue of tumor bearing rats treated with DIM@CS-NP 0.5, 1 & 2 mg/kg BW (F, G & H showing) showed almost normal architecture of mammary tissue.

### 4. Discussion

In both developed and developing countries, breast cancer seems to be a major health problem [32]. Now a day in the area of medicine nanotherapy is a hotspot, in predominantly nanoparticles supported drug delivery for cancer therapy is spreading quickly which can overcome the restrictions of conventional drug delivery system. The drug carriers of nanomaterials are highly acquired and stable high carrying capacity of optimum size and shell characteristics. Furthermore, the possibility of amalgamation of both hydrophilic and hydrophobic substances and feasibility of variable routes of administration consent to controlled drug release from the matrix and enhanced drug bioavailability [33]. Studies of DIM pharmacokinetics reveal dose dependent absorption and nonlinear increased in C_{max}, indicative of systemic absorption saturation [34,35]. However, stability enhancing micro-encapsulated formulations of DIM with extended released provides increased bioavailability suggestive of solubility limited absorption [35,36].

Moreover, in the present study investigated, the chemotherapeutic potential of DIM@CS-NP on DMBA induced rat mammary carcinoma.

### Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (mM/100 g wet tissue)</th>
<th>SOD (U*/mg ptn)</th>
<th>CAT (U**/ pg ptn)</th>
<th>GPx (U***/ mg ptn)</th>
<th>GSH (mg/100 g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.68 ± 0.14a</td>
<td>14.26 ± 1.22a</td>
<td>53.83 ± 3.55a</td>
<td>15.01 ± 0.94a</td>
<td>13.42 ± 1.00a</td>
</tr>
<tr>
<td>DMBA</td>
<td>5.60 ± 0.50b</td>
<td>8.75 ± 0.77b</td>
<td>27.18 ± 2.10b</td>
<td>7.75 ± 0.50b</td>
<td>7.15 ± 1.22b</td>
</tr>
<tr>
<td>DMBA + DIM 5 mg/kg BW</td>
<td>3.81 ± 0.23c</td>
<td>10.52 ± 0.10c</td>
<td>44.20 ± 3.33c</td>
<td>10.66 ± 0.97c</td>
<td>10.32 ± 0.91c</td>
</tr>
<tr>
<td>DMBA + DIM 10 mg/kg BW</td>
<td>3.11 ± 0.27d</td>
<td>12.88 ± 1.11d</td>
<td>51.11 ± 3.54d</td>
<td>13.94 ± 0.91d</td>
<td>11.61 ± 1.02d</td>
</tr>
<tr>
<td>DMBA + DIM 20 mg/kg BW</td>
<td>3.08 ± 0.20e</td>
<td>12.91 ± 1.22d</td>
<td>51.16 ± 3.98d</td>
<td>13.97 ± 0.96d</td>
<td>11.74 ± 0.54d</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 0.5 mg/kg BW</td>
<td>2.79 ± 0.19f</td>
<td>13.78 ± 0.89f</td>
<td>52.68 ± 4.54f</td>
<td>14.03 ± 1.33f</td>
<td>12.27 ± 1.11f</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 1 mg/kg BW</td>
<td>2.77 ± 0.18g</td>
<td>13.81 ± 0.96g</td>
<td>52.72 ± 3.65g</td>
<td>14.72 ± 1.22g</td>
<td>12.32 ± 1.28g</td>
</tr>
<tr>
<td>DMBA + DIM@CS-NP 2 mg/kg BW</td>
<td>2.75 ± 0.17h</td>
<td>13.83 ± 1.26h</td>
<td>52.75 ± 4.10h</td>
<td>14.74 ± 1.41h</td>
<td>12.79 ± 1.23h</td>
</tr>
<tr>
<td>DIM 20 mg/kg BW</td>
<td>2.74 ± 0.21i</td>
<td>14.20 ± 1.46i</td>
<td>53.79 ± 4.05i</td>
<td>14.98 ± 1.44i</td>
<td>13.32 ± 0.85i</td>
</tr>
<tr>
<td>DIM@CS-NP 2 mg/kg BW</td>
<td>2.71 ± 0.20j</td>
<td>14.24 ± 0.91j</td>
<td>53.80 ± 4.32j</td>
<td>15.00 ± 1.43j</td>
<td>13.37 ± 1.06j</td>
</tr>
</tbody>
</table>

U*: Amount of enzyme to inhibit 50% NBT reduction/min.
U**: μmol of H2O2 consumed/min.
U***: μg of GSH consumed/min.

Values not share a common superscripts differ significantly at a p- value of < 0.05 (DMRT).

Values are expressed as mean ± SD for six animals in each group.

In the present analysis, the food and water intake in different investigations groups were found to be unaltered. The total body weights of the cancer-induced group II animals were declined when compared to control groups. This may be due to the energy modify metabolism during tumor formation and in addition an increased level of lipid peroxidation plays an imperative role in the initiation of tumor development and in the reduction of body weight [40]. Increased body weight was observed in DIM@CS-NP treated groups compared to group II. This clearly indicates that DIM@CS-NP inhibited LPO as extended level due to the antioxidant activity of DIM@CS-NP.

There is a dynamic balance linking between the amount of free radicals produced in the body and antioxidant defense system that extinguish or scavenge them and protect their body against the harmful effects. Antioxidants may defend against the toxicity of reactive oxygen species (ROS) by the impediment of ROS formation [41]. The analysis of antioxidant levels is measured essential as they neutralize oxygen-free radicals [42]. Life threatening disease development is linked to the availability of these antioxidants [43]. A previous study showed that the augmented level of TBARS in tumor cells is associated through overproduction of free radicals and provide as an index to assess the size of tissue damage [44]. In the current study, we observed increased levels of TBARS in DMBA-induced animals. On the other hand DIM@CS-NP administration changed the lipid peroxidation status significantly indicating its anti-lipid peroxidative property.
Fig. 2. Histology on mammary tissues of control (A), DIM 20 mg/kg BW control (I) and DIM@CS-NP 2 mg/kg BW (J) rats showing normal ductal epithelial architecture of the terminal epithelial buds; mammary tissues of DMBA (25 mg/kg BW) induced rat (B) showing invasive ductal carcinoma with abnormal cellular proliferation and infiltrated ducts; Mammary tissues of tumor bearing rats treated with DIM 5 mg/kg BW (C) showing non invasive ductal carcinoma; Mammary tissues of tumor bearing rats treated with DIM 10 & 20 mg/kg BW (C & D) showing moderate ductal hyperplasia; Mammary tissues of tumor bearing rats treated with DIM@CS-NP 0.5, 1 & 2 mg/kg BW (F, G & H showing) showed almost normal architecture of mammary tissue. I, J Groups showed normal architecture of mammary tissue.
dependent manner, which protect cells and tissues from cause of cancer as compared to free DIM. This suggests that an antioxidant effect of DIM@CS-NP has a level-headed impact on cancer treatment. The study also clearly demonstrated that, among three doses (0.5, 1, 2 mg/kg BW) of DIM encapsulated chitosan nanoparticles 0.5 mg/kg BW increased antioxidant levels and trim down lipid peroxidation levels in plasma, liver and mammary tissues [46].

Histopathological assessment of mammary tissue of cancer bearing animals showed carcinomas exhibited invasive ductal carcinoma. In contrast, DIM@CS-NP treated rats exposed no sign of cellular proliferation and necrosis. Pronounced effect was observed in 0.5 mg/kg BW DIM@CS-NP treated animals by the normal architecture of mammary tissue. Therefore, it suggests that the DIM@CS-NP exert chemotherapeutic potential against DMBA-induced mammary carcinogenesis for targeted drug delivery and sustained release manner.

5. Conclusion

The results of the present study suggest that the DIM@CS-NP having chemotherapeutic properties against on DMBA-induced mammary carcinogenesis through the alteration of biochemical parameters, via the modulation of lipid peroxidation and enhancement of antioxidant status. Among the three doses of DIM@CS-NP were used, the lowest dose (0.5 mg/kg BW) was more effective. We anticipate cancer treatment with the DIM@CS-NP presents a new strategy in targeted drug delivery, sustained release manner. Further the nanoformulated of DIM@CS-NP is considered to be safe and effective. These preliminary results could serve as a good platform for future experimentations with DIM@CS-NP on different human cancers. However, further study is required at the molecular level to elucidate the mechanism of DIM@CS-NP in DMBA-induced mammary carcinogenesis.

Executive summary

- Bioactive compounds from plant origin have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals. Many indole derivatives are found naturally in cruciferous plants, one among the compound DIM has high antioxidant capacity and reveal chemotherapeutic in many cancer studies.
- The potential of nano formulated DIM to treat the cancer in oxidant; antioxidant and histopathological investigations are examined as a preliminary study. More work also needs to do a molecular level.
- All the above said parameters demonstrated chemotherapeutic qualities of DIM@CS-NP. Therefore, compounds from these sources hold potential for the further development of pharmaceutical products.

Author’s contributions

Dr. S. Mirunalini participated in design and coordination of this study. S. Isabella carried out the studies and drafts the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical approval

After obtaining proper approval from the Institutional Animal Ethics Committee for the Control and direction of Experimental Animal (CPCSEA approval no: 1123) guidelines.

Funding source statement

No funding source.

Acknowledgement

Dr. K. Pandian, Associate Professor, Department of Inorganic Chemistry, University of Madras, Guindy Campus, Chennai-600 025, Tamil Nadu, India for nanoparticles synthesis and characterization studies.

References
