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# Therapeutic potential of betaine and its derivatives in cancer treatment: a comprehensive review

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Bioactive natural compounds and their analogues play a significant role in the treatment of fatal diseases such as malaria, pneumonia, hepatic disorders, heart diseases and various types of cancer. Existing chemoprotective treatments for different types of cancer employ numerous natural compounds from medicinal plant extracts, fruits, vegetables, herbs, microorganisms and marine life. One of these bioactive compounds is betaine, which is a natural constituent with remarkable antioxidant and anticancer properties. The betaine scaffold and its derivatives have demonstrated suppressive effects on the progression, metastasis and angiogenesis of cancer cells. Although multiple studies have reported that betaine and its derivatives demonstrate remarkable efficiency in treating cancer or preventing tumor growth, a review summarizing the literature on this important anticancer agent is lacking to date. Thus, the present review article provides a detailed overview of the reported literature on the use of betaine and its derivatives in the prevention of different types of cancers and discusses the inhibitory activities of betaine against various types of tumorigenesis and its mechanism of action. The synergistic effects of betaine with phytochemicals and nanostructure-based medication delivery systems and its molecular modification for lowering the risk of cancer in humans are also discussed in detail.

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## 1. Introduction

Cancer has become a major health concern in recent years as it is the second leading cause of mortality after heart disease.<sup>1,2</sup> Cancer is defined by the uncontrolled growth of cells, which results in the formation of tumors.<sup>3</sup> It can result from genetic anomalies in any gene that codes for a cell cycle protein or somatic mutations in upstream cell signalling pathways. Annually, millions of patients die from cancer-related causes, despite the increasing availability of traditional cancer treatment alternatives.<sup>4</sup> Furthermore, many conventional therapeutic approaches are no longer effective against many malignant tumors because of the metastasis, recurrence, diversity, and resistance of cancer cells to chemotherapy and radiation.<sup>5</sup> Conventional cancer therapy approaches cannot always yield adequate results, since numerous limitations have been identified. Chemotherapy is considered the most popular therapeutic modality and is employed in numerous cancer therapies.<sup>6</sup> However, conventional chemotherapeutic drugs

have been widely shown in the literature to have the drawback of nonspecific distribution, resulting in low bioavailability, quick blood clearance, and relatively poor solubility in bodily fluids.<sup>7,8</sup> In addition, the emergence of drug resistance in tumor cells following repeated antitumor drug treatment significantly reduces the curative effect of chemotherapy.<sup>9</sup> The ultimate objective of cancer therapy agents is to selectively kill tumor cells while preventing damage to the surrounding healthy cells and tissues, although this has not been accomplished yet.

Herbs and botanical plants are natural medicines that have proven capacity and therapeutic qualities to inhibit cancer growth. Additionally, natural dietary ingredients such as fruits, vegetables, and spices have gained significant interest from the scientific community and general public,<sup>10</sup> and some of these have been turned into functional foods.<sup>11</sup> Dietary agents are made up of a broad range of physiologically active substances that are found in many plants and have been utilized for thousands of years in indigenous medicine owing to their strong anti-inflammatory, antifungal, antiviral and antibacterial properties.<sup>12</sup> They are also beneficial for treating or preventing systemic and infectious disorders because of immunomodulatory processes such as altered cytokine secretion, histamine release, immunoglobulin synthesis, immunoglobulin class switching, expression of cellular co-receptors, lymphocyte proliferation and stimulation of phagocytosis.<sup>13</sup>

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Generally, cancer occurs due to abnormalities in genetic and epigenetic processes. Epigenetic processes work by regulating gene expression through a variety of channels without changing the sequence of DNA. Cytosine residue methylation in cytosine-guanine (CG) pairs, which yields 5-methylcytosine, is one of the epigenetic techniques involved in DNA methylation. The loss of DNA integrity and genetic abnormalities can result from the hypomethylation of CG sites. Tumor cells may begin to grow and spread as a result of the inactivation of tumor suppressors such as p16 and activation of particular proto-oncogenes such as c-Myc.<sup>14</sup> Attaining balance between DNA methylation and demethylation mechanisms sustain optimum methylation patterns under normal physiological settings. However, this balance is upset by several pathogenic phenomena such as cancer, inflammation and oxidative stress. Due to its reversible nature, the process of DNA methylation is gaining interest as a potential target for the treatment of cancer. According to certain research, nutrition and diet can influence DNA methylation by promoting the synthesis of methyl donors at particular CG sites. The field of nutri-epigenomics, the interface between nutrients and epigenetics, is one that shows promise for the application of bioactive foods in cancer treatment in the future. These bioactive foods are regarded as necessary methyl donors for DNA methylation and include vitamin B12, folic acid, choline and betaine.<sup>15</sup> Therefore, they can have both curative and preventive effects on cancer.

In this review article, we first briefly discuss the anticarcinogenic action of betaine and its different derivatives and complexes (Fig. 1), particularly focusing on current investigations analysing their impact on the development of cancer and their possible underlying mechanisms. After that, we discuss novel approaches to treat cancer by combining betaine with anticancer medications, phytochemicals, nanotechnology and structurally modified betaine molecules.

## 2. Production of betaine and its significance

Betaine, also known as trimethyl glycine, was initially discovered in beets (*Beta vulgaris*) in the 19th century.<sup>16</sup> It is a stable, harmless natural chemical found in plants, animals and microorganisms. In addition, it is present in high amounts in microbes, aquatic invertebrates and other dietary sources such as spinach, wheat bran and wheat germ. Choline metabolism produces betaine (Fig. 2) endogenously or it can be obtained exogenously through the diet.<sup>17</sup> Betaine can prevent numerous diseases such as kidney malfunctioning, liver disorders, homocysteinemia and heart problems and has a wide range of physiological benefits on overall health. Betaine, similar to other electrolytes, functions as an osmolyte by donating methyl groups to maintain the intracellular osmotic pressure. The structure and function of proteins are stabilized when cells regulate the surface tension of water through the action of betaine, which exhibits minimal or no binding to protein surfaces. Thus, it defends against osmotic stress on proteins,

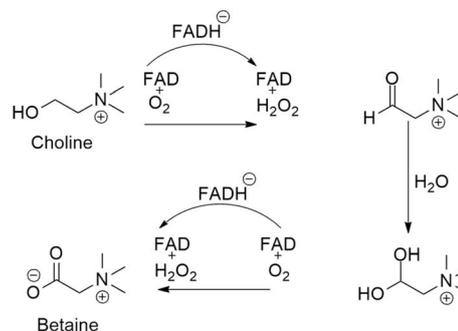


Fig. 2 Production of betaine from choline metabolism.

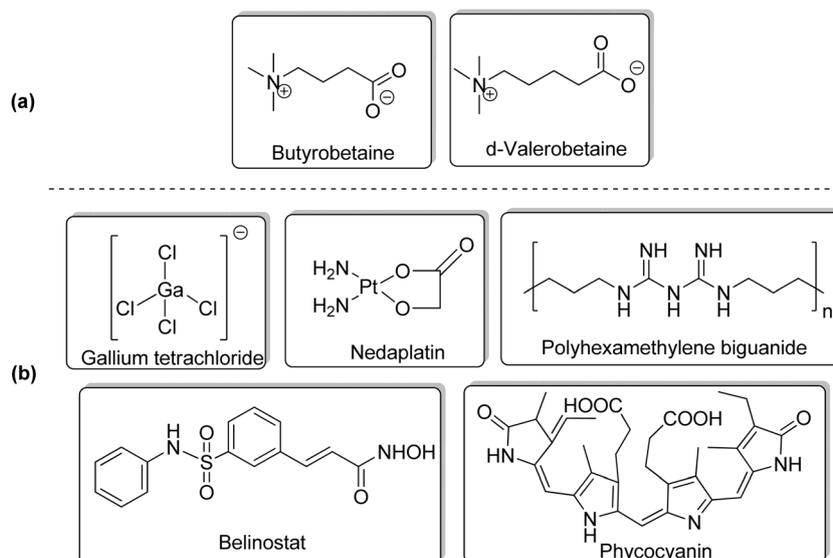


Fig. 1 Some common (a) derivatives and (b) composites of betaine exhibiting potent anticancer activities.



cells, and enzymes. Because the kidney has a high concentration of urea and electrolytes, this is especially important. In terms of hydrating albumin, betaine is the most successful osmolyte that has been researched. It is capable of preserving haemoglobin solvation and creating nearly a full monolayer of water surrounding proteins. Betaine, being an osmolyte, prevents the immune system from being affected by the hyperosmolarity-induced inhibition of tumor necrosis factor  $\alpha$ -release, prostaglandin synthesis, and cyclooxygenase-2 expression in liver-resident macrophages, known as Kupffer cells. The ability of betaine to donate its methyl group to the harmful metabolite homocysteine and convert it to methionine is one of its other significant roles. Betaine-homocysteine methyltransferase (BHMT), an enzyme first believed to be mostly found in the liver and kidneys, is responsible for catalyzing this process. A recent study has shown that BHMT is expressed in the gut and white adipose tissue, two significant organs. These results have encouraged further investigation into the potential benefits of betaine for both human wellness and the prevention of disease.

### 3. Chemical aspects of betaine in different types of cancer treatment

Betaine is an inert, naturally occurring chemical that has three extra methyl groups. Studies on a variety of human illnesses such as cancer, Alzheimer's disease and metabolic syndrome revealed the advantageous and beneficial effects of betaine. Furthermore, in *in vitro* tests on cancer, betaine has been shown to suppress the proliferation of cancer cells. According to one hypothesis, betaine regulates oxidative stress, apoptosis and inflammation in single-carbon metabolism, which is effective in cancer treatment. Unusual alterations in DNA methylation contribute to the initiation and advancement of cancer by triggering the activation of specific proto-oncogenes such as c-Myc and the inactivation of specific tumor suppressors such as p16. BHMT catalyzes *trans*-methylation activity, which requires betaine as a methyl group donor. It was revealed that the methylation levels were influenced by the presence of methyl group donors. Additionally, the *trans*-sulfuration pathway, which changes homocysteine into cystathionine, is connected to the methyl group donors. The level of glutathione (GSH) is one of the key indicators of the oxidant-antioxidant state in these pathways.<sup>18</sup> *Trans*-methylation and *trans*-sulfuration pathway metabolite level alterations have been identified as initiators of oxidative stress and apoptosis. The amounts of oxidant-antioxidant molecules and methionine cycle metabolites were altered by varying betaine doses. Low-dose betaine administration increased the GSH and S-adenosyl methionine (SAM) levels, while lowering the homocysteine levels. An essential transcription factor called nuclear factor- $\kappa$ B (NF- $\kappa$ B) regulates a large number of genes linked to oxidative damage-induced inflammation. Pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are encoded by these genes. When associated with certain cancer disorders, betaine can change these cytokine levels. Cancer is

caused by unchecked cell division and the inability of cells to undergo apoptosis.<sup>19</sup> Therefore, substances that can trigger the death of cancer cells can lead to the development of effective cancer treatment medications. Caspases, which are primarily engaged in the mechanism of apoptosis, are impacted by betaine. In following sections, the progress in exploring anti-cancer potential of betaine (Table 1) thus far is described in detail.

#### 3.1 Suppression of breast cancer

Breast cancer is the most common type of cancer in women worldwide. The World Health Organization (WHO) states that breast cancer is the most common proliferative disease among women globally, which accounts for up to one-third of newly diagnosed cases of women cancer in some countries.<sup>36</sup> Annually, more than 1.5 million women (or 25% of all women with cancer) are diagnosed with breast cancer worldwide.<sup>37</sup> It was estimated that breast cancer contributed 30% of all new cancer diagnoses among women in the United States in 2017.<sup>38</sup> Breast cancer is usually metastatic because it spreads to distant organs such as the liver, brain, lungs, and bone, making it incurable. One of the biggest challenges in its effective treatment is the resistance of breast cancer cells to the current chemotherapy treatments. This disease, once it has spread to distant areas, is typically incurable with current systemic therapies including radiation, chemotherapy, immunotherapy, and hormone therapy. Thus, it is necessary to create novel approaches for the early identification, management, and elimination of cancer resistance.<sup>39,40</sup>

Betaine, a metabolite of choline,<sup>41</sup> has been found to be an effective cancer-preventive agent. Increased betaine and choline consumption have also been shown to decrease the mortality associated with breast cancer in a dose-dependent manner, suggesting that this might be a potential way to either prevent it from developing or to help lower its mortality once it has been identified.<sup>42</sup> Some complexes of betaine, focusing on the recent developments in breast cancer therapy and its identification, are discussed below (Fig. 3).

**3.1.1 Betaine-nanoparticle synergy in breast cancer treatment.** The impact of betaine gallium-tetrachloride (BTG), a newly synthesized drug, either alone or in combination with ZnO nanoparticles (BTG + ZnO-NPs) was studied on the occurrence of mammary tumors in female rats produced by 7,12-dimethylbenz-anthracene. X-ray crystallography was used to determine the molecular and crystal structure of the prepared BTG. In an *in vitro* study, BTG was shown to be more cytotoxic than BTG + ZnO-NPs on the human breast cancer (MCF-7) cell line. An *in vivo* investigation revealed that the tumor-bearing rats (DMBA group) had a blood antioxidant status that was noticeably lower than normal. This was indicated by the considerable drop in glutathione (GSH) content, glutathione peroxidase (GPx), superoxide dismutase (SOD), and CAT activities, all of which were linked to a noticeably elevated malondialdehyde (MDA) content. Compared to the control, both treatments resulted in remarkably higher CAT and SOD activity and a corresponding drop in MDA levels. The results also demonstrated that the caspase-3 enzyme and apoptosis in the



Table 1 Different synthetic and natural derivatives of betaine explored thus far for cancer treatment

Str. no.	Type of cancer	Betaine and its complexes	Cell line and animal model	Observed effects	Ref.
1	Breast cancer	Betaine gallium tetrachloride–ZnO NPs Pegylated chitosan nanoparticles loaded with betaine and nedaplatin Polyhexamethylene biguanide–betaine (PHMB–B) + silver sulfadiazine	Mammary tumors in female rats Mammary gland tumors in rats Breast cancer wounds in woman	Apoptosis increased Lowering of MCF-cell line viability and reduction in the size of tumors Biofilm treatment	20 21 22
2	Lung cancer	Nanoemulsion of betaine and belinostat containing BGS Synergistic effect of betaine and C-phycocyanin Betaine and C-phycocyanin as micro nutrients	ER-positive ZR-75-1 and TNBC, MDA-MB-231 A549 cell line of nude rats A549 cells in naked rats	Decrease in angiogenesis-related indicators, <i>i.e.</i> VEGF and TSP-1 Decrease in NF- $\kappa$ B expression and increase in proapoptotic p38 MAPK and cell cycle arrest in the G2/M phase Decrease in phosphorylated AKT/total AKT ratios, while increase in phosphorylated p38/total p38 ratio, increase in autophagy and apoptosis	23 24 25
3	Cervical cancer	Betaine	MCF-10A and HeLa cells	Interruption of cell cycle at the G1/S or S/G2 checkpoints, activation of p53, cyclin D1, and apoptosis	26
4	Colon cancer	Betaine	<i>In vivo</i> tests on mice	Reduction in inflammatory cytokines, including TNF- $\alpha$ , IL-6, iNOS, and COX-2	27
5	Prostate cancer	Betaine	DU 145 cell line	Decrease in glutathione GSH and total antioxidant status (TAS), cell proliferation and increase in apoptosis	28
6	Oral squamous cell carcinoma	Betaine	C4-2B cell line	Upregulation of the protein expression of Bax, caspase-3 and decrease in phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT), and nuclear factor (NF- $\kappa$ B)	29
7	Hepatic cancer	Synergistic effect of $\delta$ -valerobetaine and $\gamma$ -butyrobetaine Betaine	CAL 27 cell line Rat liver cells	Activation of SIRT1 and decrease of cyclin B1 and procaspase 3 Activation of proto-oncogenes, <i>i.e.</i> , <i>c-myc</i> and inactivation of tumor suppressors genes, <i>i.e.</i> , <i>p16</i>	30 31
<b>Role of betaine in drug delivery systems</b>					
i	Breast and cervical carcinoma	Betaine complex with p-SC4	MCF-7 and HeLa cells	Cytotoxicity	32
ii	Chronic myeloid leukemia	Betaine–dasatinib complex		Decrease in pH of the gastrointestinal tract	33
iii	Chronic lymphocytic leukemia and non-Hodgkin's lymphoma	Dual chlorambucil-tailed betaine conjugate	MCF-7, HeLa and HepG2 cancer cells	Cytotoxicity	34
iv	Multiple cancers	DOX–SPBB–siRNA	A549 cells	Serum tolerance, low cytotoxicity and impressive gene delivery	35



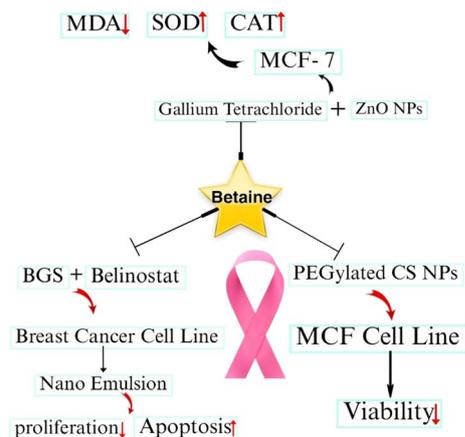


Fig. 3 Some complexes of betaine, focusing on the recent developments in breast cancer therapy and its identification.

mammary glands were markedly activated by both treatments. Compared to the DMBA group, the treatment was observed to dramatically lower the plasma iron and iron-binding capacity in tumor-bearing rats. The anticancer impact of BTG alone was found to be as potent, and even more efficient in certain aspects than that of BTG + ZnO-NPs, indicating that a combination is not necessary. Thus, the newly developed synthetic gallium compounds may offer new hope for the advancement of breast cancer treatments<sup>20</sup>

Breast adenocarcinoma (MCF-7) cells, as the most commonly used breast cancer cell line<sup>43</sup> in cancer research, and breast cancer-bearing rats were used to examine the anticancer effects of PEGylated chitosan nanoparticles (CS-NPs) coloaded with betaine (BT) and nedaplatin (ND). Nanoparticles of different polymers are used as vehicles for drug delivery due to their ability to encapsulate different active compounds and modify their surface.<sup>44,45</sup> In this case, due to the bioavailability and biodegradability of chitosan, it is mainly used for the synthesis of nanoparticles as effective drug delivery vehicles.<sup>46</sup> PEG-uncoated and PEG-coated CS NPs covering either BT, ND, or both (BT-ND) were synthesized using the ionotropic gelation technique. The produced nanoparticles had comparatively high surface charges. Due to their spherical shape, they showed high entrapment efficiency. The results of release studies showed that both PEGylated and non-PEGylated CS NPs could release their contents into the pH 5.5 micro-environment of tumor cells. Furthermore, the NPs showed exceptional capacity to reduce the viability of MCF-7 cells. It was discovered that BT-ND/PEG-CS NPs had the greatest potency among the NP preparations, resulting in a 90% reduction in the size of the tumors in the mammary gland of rats compared to the animals attached treated with the vehicle<sup>21</sup> (Table 2).

### 3.1.2 Healing power of betaine in breast cancer wounds.

Wound healing can be inhibited by the formation of a biofilm in the wound base,<sup>53</sup> which can lead to a chronic infection and an ongoing immunological response in the patient. Because biofilms have tough outer layers that are difficult to break through,

Table 2 Properties of betaine-nanoparticles

	Polymeric nanoparticles (PNPs)	Liposomes	Metal-organic frameworks (MOFs)
Physicochemical properties	Particles of solid colloids, ranging in size from 1 to 1000 nm, made of biodegradable polymers	Biocompatible and biodegradable spherical vesicles containing lipid bilayers, ranging in size from 50 to 450 nm	90–300 nm in size, crystalline porous structures are made up of organic linkers and metal ions
Drug-loading capacity	High loading for hydrophobic medications: regulated release characteristics	Drugs that are hydrophilic or hydrophobic can be encapsulated with an efficiency of up to 92%	Significant drug loading is possible due to the high surface area; encapsulation efficiency is approximately 90%
Pharmacokinetics	Reduced rates of degradation and increased bioavailability	PEGylation enhances stability; the reticuloendothelial system (RES) clears it quickly; short half-life	Varies; relies on metal ions and linkers; some show quick clearance, while others show longer circulation
Toxicity profile	Low toxicity; depending upon breakdown products and polymer type	Generally safe, although susceptible to oxidation and leaks	The discharge of metal ions may be hazardous; composition affects biocompatibility
<i>In Vitro</i> efficacy	Elevated rates of apoptosis in cancer cell lines, long-term medication release	Increased cancer cell cytotoxicity and improved cellular uptake	Effective medication release in acidic environments with dual-responsive release (pH/ultrasound)
<i>In Vivo</i> efficacy	Longer survival rates and a notable reduction of tumor development in animal models	Enhanced permeability and retention (EPR) impact for better tumor targeting and decreased systemic toxicity	Quick absorption with a long half-life; biodegradable with low toxicity
<b>Examples</b>	Betaine coloaded with PEGylated chitosan nanoparticles (CS NPs) Nanoparticles based on poly( <i>t</i> -butyl betaine carboxylate)	Liposome-like nanocapsules of dual-tailed betaine Liposomal form of beet root pigment	Ag@NMOF nanocomposite HAP/MIL-88(Fe) (HM) nanocomposite
Ref.	21, 47 and 48	34, 49 and 50	51 and 52



antibiotics and antiseptics might not work as intended. In this case, for the treatment of biofilms, polyhexamethylene biguanide–betaine (PHMB–B) has been recognized as an efficient antiseptic agent. Kim *et al.* described an effective and infection-free flap covering achieved in a patient with advanced breast cancer with the use of PHMB–B and silver sulfadiazine in the management of chronic wound biofilms. Following the diagnosis of breast cancer in a 46 year-old woman, she developed chronic sores, which had gone untreated for two years. Biofilms intensified the chronic wounds following radiation and chemotherapy. Surgical debridement<sup>54</sup> and povidone-iodine dressings were applied after the palliative complete. However, the wound remained painful and did not show any signs of healing. Silver sulfadiazine ointment was substituted for the povidone-iodine dressings; however, this did not help to manage the biofilm. After applying PHMB–B in addition to silver sulfadiazine, the wound improved and no detectable microbes were found. After that, a transverse rectus abdominis myocutaneous flap was executed on the pedicle basis without any issues. Consequently, in patients with advanced breast cancer, PHMB–B and silver sulfadiazine together can successfully remove biofilms.<sup>22</sup>

**3.1.3 Betaine, bacteria, and belinostat fusion in breast cancer.** Breast cancer has increased the fatality rate of cancer globally. In this case, Sara *et al.* attempted to decrease the toxicity, while improving the possibilities for treating breast cancer through the targeted delivery of several medications both individually and in combination. Utilizing expelled bacterial cells, also referred as bacterial ghosts (BGs), for drug administration is a viable approach to address inadequate drug delivery. The goal of their study was to create a novel complex delivery system using BGs of the *Salmonella typhimurium* ATCC 1403 strain, which has been extensively refined and its safety verified. These BGs were loaded with a class II HDACI (belinostat) and linked to nano-emulsion droplets of betaine. Two cell lines were used for testing the newly developed complex delivery method of BGs, *i.e.*, ER-positive ZR-75-1 and TNBC MDA-MB-231, respectively. Their results showed a combination index (CI) of <1 using the WST-1 proliferation assay, demonstrating the synergistic impact between BGs loaded with belinostat per constant count of BGs CFU mL<sup>-1</sup> and betaine nano-emulsion at low doses with a polydispersity index of less than 0.5. The combined 72 h treatment significantly inhibited the cell viability and apoptosis induction in both the tested MDA-MB-231 and ZR-75-1 cells compared to treatment with either betaine or belinostat alone. Furthermore, following a 24 h treatment period, the obtained delivery revealed a substantial decrease in the levels of angiogenesis-related indicators such as VEGF and TSP-1 at dosages of 1 mM betaine nano-emulsion and 0.2 μM belinostat. Thus, this was proven to be a novel BG delivery system with the combined synergistic mechanistic action of two distinct anti-cancer drugs as a potentially effective way to prevent the angiogenesis and proliferation of breast cancer cells.<sup>23</sup>

### 3.2 Suppression of lung cancer

Globally, lung cancer is the leading cause of cancer-related mortality.<sup>55</sup> Lung cancer appears in two varieties, non-small

cell lung cancer (NSCLC) and small cell lung cancer. Between them, the former is the most common,<sup>56</sup> accounting for about 85% of lung cancer cases and surgery, immunotherapy, tyrosine kinase inhibitors, and platinum-based chemotherapy are all used to treat it.<sup>57</sup>

**3.2.1 Synergistic anticancer potential of betaine and C-phycoerythrin in lung cancer treatment.** The combined effects of betaine and C-phycoerythrin (C-PC) were studied on the growth of the lung cancer A549 cell line both *in vivo* and *in vitro*. In case of betaine, there was a 50% decrease in cell viability, whereas in the case of C-PC treatment alone, there was no reduction in cell viability. However, there was a 60% reduction in cell viability when betaine and C-PC were co-cultured. The combined treatment decreased the TNF-α-induced activation of NF-κB expression and increased the levels of proapoptotic p38 MAPK. Remarkably, for almost 60% of cells, combination therapy resulted in a cell cycle arrest in the G2/M phase. A549 cells were injected into the right flank of pathogen-free male nude rats for use in *in vivo* experiments. Rea *et al.* reported that the tumor weights and volumes were much lower in the groups treated with either betaine or C-PC.<sup>58</sup>

**3.2.2 Micronutrient tumor control.** Carmen *et al.* also explained that betaine and C-phycoerythrin are micronutrients that have antiproliferative, antioxidant and anti-inflammatory effects. They studied the nutritional effects of these two micronutrients on the growth of the A549 cell line in nude rats, and also determined their underlying mechanism. They reported that tumour growth was decreased due to these two nutrients when supplied together or separately through processes such as tumor expression of proinflammatory cytokines (IL-1, Cox-2, and TNF-), NF-κB activation, and an increase in lipid peroxidation. Furthermore, several processes were unique to each micronutrient or to their combination. The phosphorylated AKT/total AKT ratios decreased, while the phosphorylated p38/total p38 ratios increased in response to C-phycoerythrin, both of which supported autophagy<sup>38</sup> and apoptosis. Conversely, betaine linked to C-phycoerythrin elevated the ratio of caspase-3 to pro-caspase-3. Additionally, how the development of implanted A549 cells in nude rats was impacted by their voluntary physical activity was also studied. It was demonstrated that engaging in voluntary physical exercise inhibited the formation of lung tumors due to the same mechanisms as that of betaine and C-phycoerythrin, *e.g.* increase in lipid peroxidation, the activation of NF-κB and p38, and the inhibition of AKT. Betaine and C-phycoerythrin-enriched diets inhibit the proliferation of adenocarcinoma in the lung cells, revealing a potential anticancer effect.<sup>25</sup>

### 3.3 Suppression of cervical cancer

Cervical cancer accounted for 9% of all new cases and 8% of all female cancer deaths in 2008, making it the third most common cancer overall and the fourth leading cause of cancer-related death in women worldwide.<sup>59</sup> The majority of these cases, *i.e.*, more than 85%, occur in underdeveloped nations. Additionally, 27% of all cervical cancer fatalities occur in India, the second most populous nation in the world.<sup>60</sup> The lack of screening for



the early diagnosis of cervical cancer is a major contributing factor to the disproportionately high incidence of cervical cancer in medically underprivileged populations and developing nations.<sup>64,62</sup> Cervical cancer has been treated with a comprehensive approach, which includes chemotherapy, radiation therapy, and surgery since 1999.<sup>63</sup> Many phytonutrients extracted from edible plants are the most promising agents that have been shown to disrupt various phases of carcinogenesis, potentially reducing 7–31% of all cancer cases globally.<sup>64</sup> One of them is betaine, which inhibits the methylation patterns of proto-oncogenes and tumor suppressors to control their expression and has an anticancer effect.<sup>65</sup>

Originating from the biopsy of a tumor of cervical cancer, the first human cell line to be developed in culture was HeLa cells.<sup>66</sup> The underlying molecular processes responsible for the impact of betaine on HeLa cell proliferation and apoptosis was studied. The anticancer activity of betaine in HeLa cells was assessed at concentrations of 0.1, 1.0, 5.0, 20.0, and 100.0 mg mL<sup>-1</sup>, respectively. MCF-10A was also found to be a normal diploid cell control. Using the MTT test, Yu *et al.* discovered that exposure to increasing betaine levels significantly decreased the growth of HeLa cells ( $p < 0.05$ ). High concentrations of betaine (>5.0 mg mL<sup>-1</sup>) greatly accelerated the death of HeLa cells, and the percentage of S phase cells in the low dosage groups (<5 mg mL<sup>-1</sup>) was significantly higher than in the high dose groups, with the rates of the sub-G1 phase being the opposite ( $p < 0.01$ ). The low-dose groups SOD activity was somewhat greater than that of the control group. The expression of the apoptosis-suppressive gene Bcl-2 and the apoptosis-promoting genes BAX, P53, and caspase-3 showed clear synchronization and association. When the cell cycle gets interrupted at the G1/S or S/G2 checkpoints, p53 and cyclin D1 may be activated in response to a stimulus that induces apoptosis.<sup>26</sup>

### 3.4 Suppression of colon cancer

Colon and rectal cancers are types of cancerous growth originating from the lining of the large intestine. Individuals who suffer from Crohn's disease (CD) and ulcerative colitis (UC) are those with inflammatory bowel disease (IBD) who are more susceptible to be diagnosed with colorectal cancer (CRC).<sup>67</sup> CRC is a global health issue whose prevalence is steadily rising. Up to 20 years ago, IBD was thought to be rare in Asia.<sup>68</sup> However, more recent cohorts from referral centres and population-based studies have revealed an increase in the incidence and prevalence of IBD in Asia.<sup>69</sup> A defective immune response and luminal, environmental and genetic variables, such as tumor necrosis factor (TNF)- $\alpha$ , can contribute to the development of IBD. Particularly, the blood, intestinal mucosa, and faeces of IBD patients had the indicated level of TNF- $\alpha$ . Other pro-inflammatory mediators have also been found to be elevated in the faeces and rectal dialysates of IBD patients, in addition to TNF- $\alpha$ .<sup>70</sup> Based on available data, there is a growing trend of TNF- $\alpha$  inhibitor use in IBD-related research and patient populations. TNF- $\alpha$  antibodies, such as chimeric IgG1 monoclonal antibody infliximab, human monoclonal IgG1 antibody adalimumab, and PEGylated Fab fragment of a humanized IgG4

isotype monoclonal antibody certolizumab pegol are the most often utilized biologics in IBD.<sup>71</sup> However, these anti-TNF medications explicitly increase the overall risk of serious infections such as varicella, tuberculosis, and other diseases.<sup>72</sup>

Researchers have demonstrated that betaine exhibits anti-inflammatory properties by suppressing reactive species (RS) and adjusting the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) during the aging process, as evidenced by both *in vitro* and *in vivo* investigations.<sup>73–75</sup> Likewise, Dong *et al.* investigated the impact of the anti-inflammatory and tumor-preventive properties of betaine on mice with colitis-associated cancer. They used azoxymethane (AOM) and dextran sulfate sodium (DSS) to create colon cancers in mice during the *in vivo* tests, and they assessed the impact of betaine on tumor formation. When betaine was administered, the incidence of tumor formation was considerably reduced and inflammation was diminished. In the colonic mucosa, betaine treatment reduced the amount of GSSG and the production of ROS. The treatment of betaine reduced inflammatory cytokines, including TNF- $\alpha$ , IL-6, iNOS, and COX-2, according to the qPCR findings. Betaine administration reduced LPS-induced NF- $\kappa$ B and inflammatory-related cytokines in RAW 264.7 murine macrophage cells *in vitro*. Thus, it was proven that betaine is a potential treatment option for colon inflammation.<sup>27</sup>

### 3.5 Betaine for prostate cancer control

Prostate cancer (PCa) is the fifth most common cause of cancer-related mortality in males and the second most common solid tumor. Giorgio *et al.* profoundly investigated the epidemiology of this cancer worldwide and inferred its risk factors based on heredity, environmental factors, metabolic diseases, dietary factors and physical activities.<sup>76</sup>

**3.5.1 Role of betaine in DU-145 carcinoma.** Abnormal methylation is a prevalent factor in the development of cancer, activating proto-oncogenes such as c-Myc and deactivating tumor suppressor genes such as p16. Betaine is a methyl donor in *trans*-methylation, which is activated by betaine-homocysteine methyltransferase (BHMT). One of other important factors is *trans*-sulfuration, which converts homocysteine to cystathionine (Fig. 4) through the cystathionine beta-synthase (CBS) enzyme. It has been suggested that varying levels of

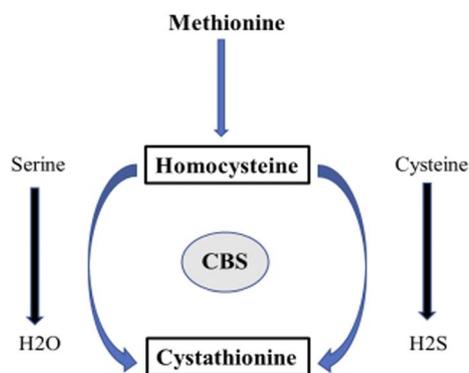


Fig. 4 Conversion of homocysteine into cystathionine.

metabolites in *trans*-methylation and *trans*-sulfuration exacerbate oxidative stress and apoptosis. As a result, low doses of betaine increased the levels of glutathione (GSH) and *S*-adenosyl methionine (SAM) and lowered homocysteine. Nuclear factor- $\kappa$ B (NF- $\kappa$ B), belonging to transcription factor family, regulates the expression of many genes related to inflammation caused by oxidative damage.<sup>77</sup> These genes carry pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin 6 (IL-6). Betaine can restore these cytokine levels in numerous cancer diseases. In addition, betaine also affects the caspase proteins, which are involved in apoptosis.<sup>27</sup>

DU-145 is an important cell line for investigating prostate cancer, and the cell viability of DU-145 human prostate cancer cells was evaluated based on dose-dependent betaine.<sup>78</sup>

The treatment employing low doses of betaine concentrations statistically enhanced the cell viability. The betaine concentration of 50 mg mL<sup>-1</sup> was the primary indicative concentration that exhibited a convincing decrease from untreated cells. Above this concentration, the cell viability greatly decreased.

Subsequently, Fatih Kar *et al.* demonstrated that betaine inhibited proliferation and caused oxidative stress and apoptosis, and its high dose treatment caused inflammation by increasing the total oxidant status (TOS) levels and decreasing the glutathione GSH and total antioxidant status (TAS) in the DU-145 prostate cancer cell line. Thus, the therapeutic effect of betaine is entirely based on its dosage and concentration.<sup>28</sup>

**3.5.2 Role of betaine in C4-2B cancer cells.** H Zhang *et al.* extensively investigated the suppression of prostate cancer tumor by the action of betaine based on its concentration. The C4-2B cell line is the part of male prostate and it is used for the examination of prostate cancer given that it has an epithelial-like morphology, making it convenient to investigate tumors.

The Bax gene is an apoptosis-promoting constituent of the bcl-2 gene group. The Bax protein governs cell death through its cooperation in the disruption of mitochondria and successive *cytochrome c* release, and is also known to be one of the essential p53 targets. Caspase-3 is well-known as one of the primary regulators of apoptosis. The activation of caspase-3 demands proteolytic processing of its dormant and inactive zymogen state into activated p17 and p19 subunits. Bax initiates the release of *cytochrome c* from the mitochondria, and as a result *cytochrome c* release stimulates caspase-3. Cleaved caspase-3 is considered to play a fundamental role in apoptosis.<sup>79</sup> This research group reported that a specific concentration of betaine upregulated the protein expression of Bax and caspase-3, and at the same time downregulated phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT) and nuclear factor (NF- $\kappa$ B).<sup>29</sup>

### 3.6 Betaine in oral squamous cell carcinoma

N D'Onofrio *et al.* substantially studied the effect of dietary betaine on the oral squamous cell carcinoma CAL-27 cell line. Oral and oropharynx cancer is challenging, precarious and escalating worldwide especially among young patients.<sup>80</sup> Oral cancer is a protruding tumor, which undergoes rapid

metastasis, and ultimately leads to death. Different oral squamous cell carcinoma (OSCC) cell lines have been employed as a tool to investigate the therapeutic treatment of oral cancer. CAL-27 is one of primary cell lines for the detailed inspection of oral cancer. This research group prudently scrutinized the synergistic role of  $\delta$ -valerobetaine ( $\delta$ VB) and  $\gamma$ -butyrobetaine ( $\gamma$ BB) (present in various diets) on the inhibition and suppression of cell proliferation and induction of apoptosis in the CAL-27 cell line. In addition, the level of sirtuins was also studied in the cell proliferation and inhibition of tumors. In mammals, there are seven members of sirtuins (SIRT1-SIRT7), among which SIRT1 plays important role in the deacetylation of histones and many nonhistone substrates. However, the complete role of SIRT1 in cancer is ambiguous in terms of whether it behaves as a tumor suppressor or tumor promoter.<sup>81</sup> Nevertheless, in the case of oral cancer, SIRT1 is highly suggested to play a role in tumor suppression.<sup>82</sup>

Furthermore, these combined betaines strikingly enhanced the generation of reactive oxygen species (ROS) as well as SIRT1, which caused deacetylation, and SIRT1 intervened in cell propagation, as clearly indicated by gene silencing with small interfering RNA. Betaines effectively induced apoptosis in CAL-27 by activating SIRT1 and degrading cyclin B1 and procaspase-3, reducing cell proliferation, and ultimately leading to apoptosis.<sup>30</sup>

### 3.7 Betaine-mediated inhibition of hepatocellular carcinoma

Hepatic cancer is one of the most common and deadliest types of malignancy, which is considered a global concern. Several factors cause liver cancer such as alcohol-induced cirrhosis, fatty liver, smoking, obesity, and hepatitis B and C virus.<sup>83</sup> The mechanism of liver cancer involves the key role of tumor-associated macrophages (TAMs). These macrophages undergo compelling phenotypic changeover during carcinogenesis. Due to their high plasticity and heterogeneity, TAMs play a significant role in the proliferation and angiogenesis of cancer cells. In a normal liver, macrophages mainly exist in form of Kupffer cells. However, with the advancement of liver cancer, pro-tumorigenic factors aggravate tissue-resident macrophages, ultimately leading to TAM inflammation and tumor progression.<sup>84</sup> A significant role of betaine was observed in the prevention of liver cancer. A study found that betaine improved liver diseases through various molecular mechanisms such as inhibiting inflammation, improving insulin resistance, reducing endoplasmic reticulum stress, decreasing oxidative stress, increasing autophagy, remodeling the intestinal flora, and regulating epigenetic alterations. Betaine can improve liver diseases by targeting signaling pathways such as NF- $\kappa$ B, AMPK, PPAR- $\alpha/\gamma$ , LXR $\alpha$ , Akt, TLR4, and caspase-3.<sup>85</sup>

**3.7.1 Mitigation of DEN-induced hepatic cancer.** Y Du *et al.* experimentally investigated the role of betaine in the amelioration of hepatic cancer. Hepatocarcinogenesis is the result of multiple considerations such as genetic and epigenetic modifications of diverse growth factors, proto-oncogenes and tumor suppressor genes. They induced liver cancer in rats by the



treatment of diethylnitrosamine (DEN), which is a potent carcinogenic agent, and evaluated the effect of betaine supplementation in the mitigation of cancer. Betaine effectively regulated proto-oncogenes such as *c-myc* as well as certain tumor suppressors such as *p16*. Studies also revealed that betaine can productively reduce lipid peroxidation and hepatic injury caused by  $\text{CCl}_4$ , hyperosmolarity and niacin.

Inactivation of *p16* and activation of *c-myc* are majorly induced by DNA methylation. The *p16* gene curtails enzymatic activity, which induces the abnormal phosphorylation of another tumor suppressor *Rb*, leading to cell proliferation. Therefore, *p16* silencing in rat liver cells was indicated by hypermethylation. Conversely, *c-myc* is crucial regulator of various cellular processes and studies revealed that the hypomethylation of *c-myc* gene results in its overexpression in hepatic cancer.<sup>86</sup> As a methyl donor, betaine effectively regulated the degree of mRNA expression of both *p16* and *c-myc*. Betaine supplementation substantially mitigated DEN-induced hepatic carcinoma.<sup>65</sup>

Moreover, a few studies investigated how BET affects the hepatitis B virus (HBV), which is the most common cause of liver illness. Southern blotting, quantitative polymerase chain reaction, and enzyme-linked immunosorbent assay were used to evaluate the anti-HBV activity of betaine both *in vitro* and *in vivo*. The therapeutic treatment of HBV is complicated by the resistance of this virus to lamivudine and interferon alpha. Thus, the impact of betaine on its resistance was examined. The findings showed that BET dramatically reduced the release of HBV DNA, HBeAg (HBV e antigen), and HBsAg (HBV surface antigen) in HepG2.2.15 cells by suppressing GRP78 expression. The serum DHBV DNA considerably decreased with betaine treatment in ducklings infected with HBV (DHBV). Betaine reduced the resistance mutation of HBV DNA (rtM204V/I) and inhibited the HBV DNA rebound caused by the resistance of this virus to lamivudine. Betaine supplementation may enhance the anti-HBV action of interferon alpha by boosting the production of antiviral dsRNA-dependent protein kinase, which is triggered by the JAK-STAT signaling pathway (JAK = Janus kinase; STAT = signal transducer and activator of transcription). These findings can offer valuable insights into the clinical utilization of betaine and HBV medication resistance solutions in anti-HBV treatment.<sup>9</sup>

### 3.8 Synthetic derivatives of betaine in chemotherapeutic transport and their molecular modeling

Chemotherapy has been employed in cancer treatment for many years, but together with this, it has many complications. If small doses of anticancer drugs are taken for treatment, they fail to get to the desired area and unable to reduce the tumor. On the contrary, high doses of chemotherapeutic drugs have been reported to damage healthy cells, making the patient susceptible to several other complications.<sup>87</sup> Moreover, the drug resistance phenomenon is another challenge encountered during the chemotherapeutic mode of treatment. Thus, several protocols have been designed to tackle the systematic toxicity, early elimination, resistance and control the release of drugs.

Strategies based on drug delivery systems (DDSs) are being manipulated to enhance the efficacy of chemotherapy with minimum side effects.<sup>88</sup> The use of numerous drug delivery vehicles to encapsulate anticancer drugs is a promising way to enable the uptake these drugs for tumor alleviation, while at the same time prevent their degradation in the circulation.<sup>89–91</sup> The host-guest complexation mode of drug delivery has attracted attention from researchers owing to its effective outcomes.<sup>92</sup>

Betaine-based DDSs (Fig. 5) are being employed to enhance the ability of anticancer drugs to treat cancer at high concentrations. The host-guest complexation of betaine with a calixarene receptor was investigated successfully for cancer treatment. Particularly, calix[*n*]arenes (*n* = 4, 6, and 8) are a compelling class of macrocycles that have been widely applied for the delivery of anticancer drugs. Calixarenes are the optimal host molecules, which are composed of phenolic units associated with methylene bridges containing an upper periphery with a para-substituent of a phenolic ring, a lower periphery with a phenolic hydroxyl group, and a hydrophobic  $\pi$  electron-rich core cavity.

**3.8.1 Synergistic effect of betaine-*p*-sulfonatocalix[*n*]arenes in cancer treatment.** *p*-Sulfonatocalix[*n*]arenes have exhibited plausible potency as carriers of chemotherapeutic drugs through host-guest complexation and augmenting their anti-cancer coaction. In a study, *p*-sulfonatocalix[*n*]arenes were shown to be harmless to human cells in *in vivo* applications up to  $10^3 \text{ mg kg}^{-1}$ . *p*-Sulfonatocalix[*n*]arenes (p-SC4) were complexed with betaine for anticancer application and the results were studied in aqueous solution for cancer treatment. Specifically, the efficacy of betaine, p-SC4 and their complex B/p-SC4 was evaluated by comparing their results. Breast adenocarcinoma (MCF-7) and cervical cancer (HeLa) were employed in the cell viability assay and cytotoxicity was evaluated by the treatment of breast adenocarcinoma (MCF-7) and cervical cancer (HeLa) with various concentrations of betaine, p-SC4 and their complex B/p-SC4. In the *in vitro* investigation, cytotoxic effects were evaluated using the SRB assay. Betaine and p-SC4 exhibited negligible cytotoxicity to cancerous cells. However, B/p-SC4 complex demonstrated remarkable cytotoxicity. The  $\text{IC}_{50}$  of the betaine/p-SC4 complex was found to be  $1.6 \pm 1.4$  and  $3.3 \pm 1.1 \text{ mg mL}^{-1}$  against MCF-7 and HeLa cells, respectively.<sup>32</sup>

### 3.9 Drug conjugation chemistry of betaine in cancer therapy

**3.9.1 Betaine-dasatinib combination therapy.** Dasatinib is an anticancer drug (tyrosine kinase inhibitor), which is administered for the treatment of Philadelphia chromosome-positive (Ph<sup>+</sup>) chronic myeloid leukemia (CML). Dasatinib is weakly basic in nature and its solubility is pH dependent, becoming significantly insoluble in water above pH 6 (solubility  $< 0.008 \text{ mg mL}^{-1}$ ).

Drug-drug interactions are a common phenomenon observed upon the simultaneous intake of different drugs. A drug inducing a change in gastric pH, particularly hypochlorhydria, results in the reduced solubility, absorption and exposure of the drug.<sup>93</sup> Thus acid reducing agents (ARAs) or proton pump inhibitors (PPIs) are taken to counter the



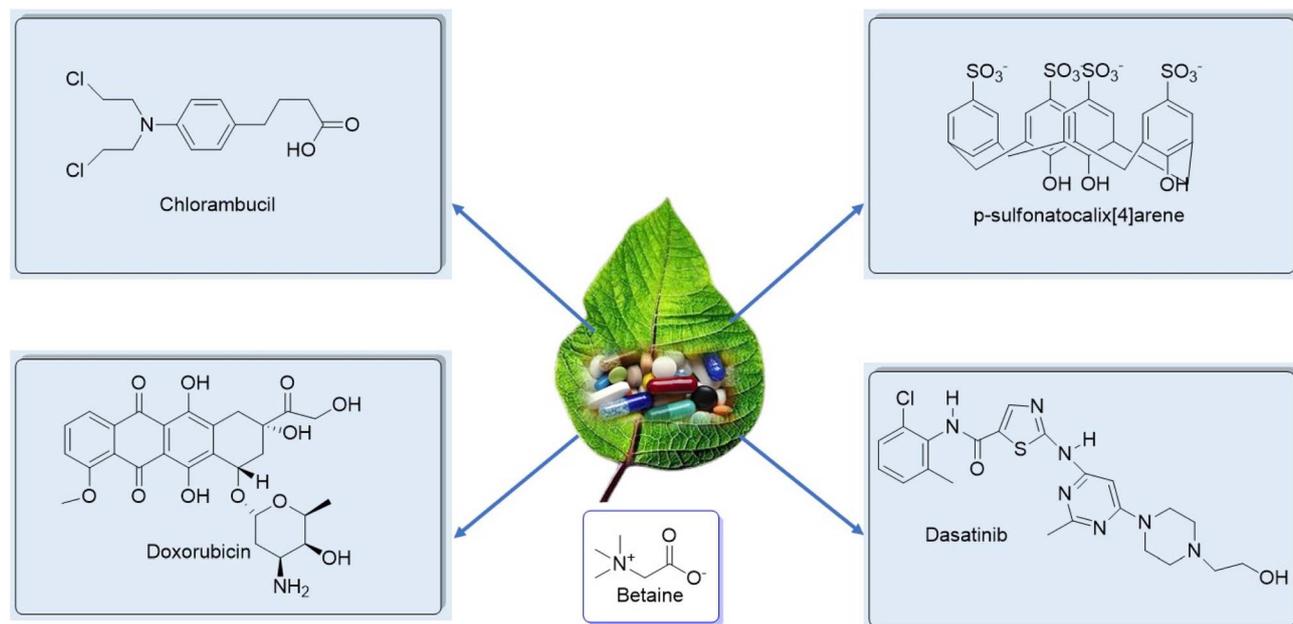


Fig. 5 Betaine-based drug delivery systems (DDSs) for cancer treatment.

symptoms of gastroesophageal reflux disease (GERD) or peptic ulcer disease. These drugs alleviate the pH in the gastrointestinal tract, hence reducing acid reflux. However, the use of these PPIs significantly impact the solubility and bio-absorption of other essential drugs.<sup>94</sup> For instance, famotidine is a PPI drug, and when it is administered with dasatinib it significantly reduces the area under the concentration *versus* time curve (AUC) and maximum plasma concentration ( $C_{max}$ ) of dasatinib by 60%. A similar phenomenon was observed in the case of rabeprazole-induced hypochlorhydria, where rabeprazole (20 mg b.i.d.) substantially lessened the  $C_{max}$  and  $AUC_{0-\infty}$  of dasatinib by 92% and 78%, respectively. Therefore, transitory gastric reacidification at the time of drug dosing may assist in alleviating gastric pH-drug–drug interactions.

Betaine hydrochloride (BHCl) is employed to mitigate this drug–drug interaction problem. BHCl is a natural supplement that is commonly used in digestive aids, which upon dosage in solid oral form gets dissociated into betaine and hydrochloric acid in gastric fluids, consequently decreasing the gastric pH and making it susceptible for dasatinib solubility. However, coadministration of BHCl predominantly enhanced the  $C_{max}$  and  $AUC_{0-\infty}$  of dasatinib by 15- and 6.7-fold, restoring them to 105% and 121%, respectively, compared to dasatinib alone. On account of this, BHCl enhanced the solubility and absorption of dasatinib with rabeprazole-induced hypochlorhydria.<sup>33</sup>

**3.9.2 Enhanced anticancer effects of dasatinib with betaine HCl and pentagastrin.** Another similar experiment was performed to analyze the absorption of the weakly basic drugs GDC-0941, dasatinib, betaine HCl, famotidine and pentagastrin. Famotidine is a drug used to reduce the gastric pH, which is known as an antacid, while pentagastrin mimics the action of gastrin hormone and augments gastric secretions. Several acid-reducing drugs, *i.e.* antacids, and proton pump inhibitors are

taken to prevent acid production and protect from peptic ulcers, while at the same time they alleviate the exposure of several weakly basic drugs such as anticancer drugs dasatinib and GDC-0941. The effect of pretreatment with pentagastrin, famotidine, betaine HCl and combined betaine HCl and famotidine was investigated on animals. The gastric pH was evaluated at regular intervals. Betaine HCl and pentagastrin worked synergistically and reduced the gastric pH. However, betaine HCl reduced the pH to 2 temporarily and it was restored. In contrary, pre-dosage of famotidine sustained the pH near 7. Famotidine reduced GDC-0941 absorption by 5-folds and dasatinib by 30-folds in contrast with pentagastrin. Betaine HCl recovered GDC-0941 exposure in famotidine-treated animals and augmented dasatinib dissolution and absorption to 1.5-fold. Betaine HCl coadministration effectively mitigated drug-induced hypochlorhydria and maintained targeted drug exposure.<sup>95</sup>

**3.9.3 Synergistic effect of betaine in chlorambucil treatment.** Chlorambucil is another anticancer drug, which is widely used for the treatment of chronic lymphocytic leukemia and non-Hodgkin lymphoma (NHL) and Hodgkin disease (types of cancer that begin in certain white blood cells that normally fight infection). To enhance the cytotoxic effect and high drug loading capacity of chlorambucil, liposomes such as nanocapsules were employed. Nanosized vesicles have been

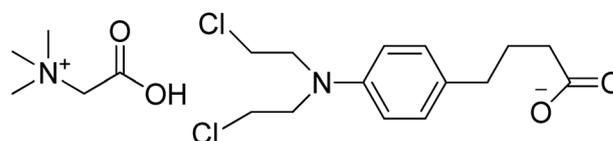


Fig. 6 Interaction between betaine and chlorambucil.



extensively adopted as drug carriers for cancer therapy, *i.e.* liposomes, micelles and nanoparticles. These methods of drug delivery enhance therapeutic advantages such as higher cytotoxicity and greater tumor selectivity.<sup>96</sup> However, they require encapsulation of drugs, resulting in an unstable drug loading. Thus, to overcome this hindrance, a new strategy was adopted by combining hydrophobic and hydrophilic ends, resulting in the formation of amphiphiles.<sup>97</sup> One of the well-known examples is the conjugation of glycine betaine and chlorambucil (Fig. 6). Betaine is a zwitterionic compound having several biological advantages. Inspired by the structure of phospholipids, the hydrophilic end of betaine and hydrophobic end of chlorambucil were combined to yield a dual-drug-tailed amphiphilic product having the maximum drug loading capability.

A dual chlorambucil-tailed betaine conjugate (DCBC) was assessed *via* both *in vitro* and *in vivo* analysis. In the *in vitro* analysis, the viability of MCF-7, HeLa and HepG2 cancer cells was evaluated using free chlorambucil and DCBC. It was reported that DCBC had high drug loading ability and enhanced cytotoxic effects compared to chlorambucil.

In the *in vivo* analysis, MCF-7 cell tumor-bearing mice were employed. After the intravenous administration of DCBC, it was reported that DCBC liposomes such as nanocapsules possess enhanced *in vivo* antitumor efficacy compared to the free drug. In addition, their high biocompatibility and safety were also reported.<sup>34</sup>

**3.9.4 Betaine-driven doxorubicin potency.** Doxorubicin (DOX) is a chemotherapeutic agent, which is used to treat various types of cancers. One of the challenges in chemotherapy is drug resistance, which can be addressed using a co-delivery system. Small interfering RNA (siRNA) is a promising tool for inhibiting gene and protein expression, where proteins responsible for drug resistance and apoptosis inhibition can be efficiently inhibited by siRNA.<sup>98</sup> However, this technology has some limitations such as reduced transfection property, rapid degradation and endosomal retention of siRNA.<sup>99</sup> This obstacle can be eliminated by using a codelivery system such polyethyleneimine (PEI), which can efficiently release chemicals from lysosomal sequestration by the protein sponge mechanism. However, PEI has also some limitations such as high toxicity and low serum stability. Therefore, chemical modification of PEI with betaine has been adopted to enhance their efficacy. The conjugation of betaine with PEI resulted in significant properties such as serum tolerance, low cytotoxicity and impressive gene delivery.

In this study, PEI-betaine (PB) was further combined with single-walled carbon nanotubes (CWCNT) to generate SWCNT-PB (SPB), which was proposed to exhibit pH-responsive lysosomal release efficacy. SPB was additionally modified by DOX, survivin siRNA and peptide BR2 (SPBB)23 to form the final product, DOX-SPBB-siRNA.

*In vitro* and *in vivo* experiments were performed using A549 cells. DOX-SPBB-siRNA considerably scaled down the tumor size in nude mice bearing A549 tumor cells, thereby exhibiting the synergistic effects of DOX and surviving siRNA without noticeable damage to normal tissues.<sup>35</sup>

**3.9.5 Sulfur-modified betaine derivatives.** Betaine-based compounds incorporating sulfur have shown potential biological activities and pharmacological efficacy<sup>100</sup> in cancer therapy to develop important drugs.<sup>101,102</sup> The addition of sulfur-containing groups such as sulfones, thiols and thioethers to scaffolds of betaine can tremendously improve its role by enhancing its metabolic stability, redox activity and increasing targeted cytotoxicity to cancer cells.<sup>103,104</sup> Sulfur atoms enable the generation of reactive oxygen species (ROS) and form a network with enzymes having a thiol group, which are critical for cancer survival and alter cancer cells to undergo apoptosis and oxidative stress. Sulfur-modified betaine derivatives have shown increased target specificity and membrane permeability, which can enhance the anticancer potency and bioavailability of betaine. In oncology, these modifications align with the current trend in medicinal chemistry of using the unique chemical profile of sulfur to enhance the efficacy and drug likeness of compounds<sup>105,106</sup> (Fig. 7).

### 3.10 Betaine as cytoprotective agents

**3.10.1 Betaine mitigating doxorubicin-induced cardiotoxicity.** Anticancer drug-induced cardiotoxicity is observed in multiple individuals having lung, ovary, breast and paediatric cancer. One of well-known anticancer drugs is the anthracycline drug doxorubicin (DOX), which is taken to treat multiple cancers. However, several studies have shown that doxorubicin causes cardiotoxicity, for example, it induces oxidative stress, which produces reactive oxygen species (ROS).<sup>107</sup> Moreover, intracellular calcium ion ( $\text{Ca}^{2+}$ ) dysregulation also generates ROS, which ultimately decreases several antioxidants, *e.g.* glutathione peroxidase and superoxide dismutase, leading to the aggregation of free radicals and causing mitochondrial dysfunction. In addition, DOX augments death receptors such as TNFR1, Fas, DR4, and DR5 at both the protein and mRNA levels. Further, treatment using DOX induces cardiac cell damage through the activation of ERK1/2, p53, and NF- $\kappa$ B dependent-PUMA (p53 upregulated modulator of apoptosis). Several mechanisms were investigated to study DOX-induced cardiotoxicity such as troponin T, sirtuins, gap junctions and calcium homeostasis.<sup>108</sup> However, there is no evident method for the treatment or prevention of this drug-induced complexity except for a few medications.

A Jaiswal *et al.* hypothesized that the beneficial effects of betaine can play a key role in the prevention of DOX-induced cardiotoxicity given that betaine is a natural, safe (9–15 g) and non-toxic methyl donor in one-cycle. It is involved in the methylation of proteins and DNA. In addition, betaine diminishes autoimmune encephalomyelitis, cortical and hippocampal MDA grading in mice.<sup>109,110</sup> It adequately reduces several brain disorders such as Alzheimer's disease. Moreover, betaine is also beneficial in metabolic disorders such as obesity by improving glucose homeostasis and decreasing hepatic steatosis. Alternatively, it plays a pivotal role in productively mitigating  $\text{CCl}_4$ -induced liver fibrosis, atherosclerosis and colitis-associated cancer. Based on all these prerequisites, this group hypothesized that betaine can be a potent natural compound to



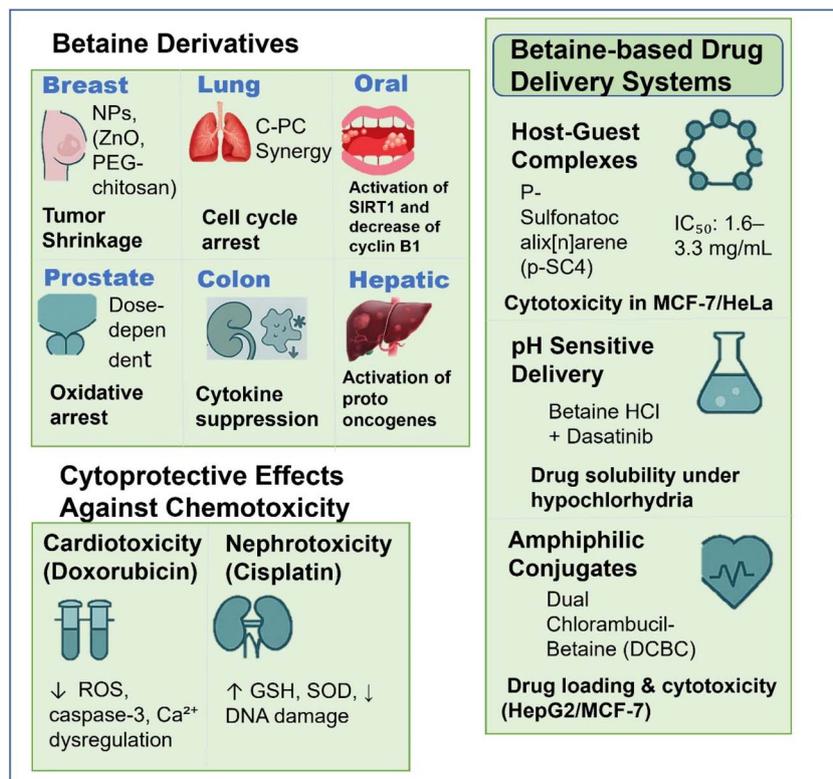


Fig. 7 Betaine derivative-based drug delivery systems.

reduce DOX-induced cardiotoxicity by inhibiting ROS production, caspase 3, oxidative stress, apoptotic factors, and Ca<sup>2+</sup> dysregulation and increasing the antioxidant level.<sup>111</sup>

**3.10.2 Betaine alleviating cisplatin-induced nephrotoxicity.** Drug-induced nephrotoxicity is one of the major causes of kidney dysfunction and impairment. Besides, chemotherapeutic drugs cause 60% incidence of renal disease. Many mechanisms are involved in anticancer drug-induced kidney damage such as hypercalcemia, tumor lysis syndrome, renal parenchyma and myeloma kidney. Consequently, the kidneys fail to detoxify the drugs and become susceptible to acute kidney injury (AKI), which is the inception of chronic renal failure.<sup>112</sup>

Cisplatin is antineoplastic agent, which is widely being employed in the treatment of various cancers such as head and neck cancer, lung cancer, cervical cancer, ovarian cancer, bladder cancer and oesophageal cancer. However, this drug is responsible for many complexities such as DNA damage and cytoplasmic organelle dysfunction notably in the mitochondria and endoplasmic reticulum.<sup>113</sup> In addition, cisplatin elevates the blood urea nitrogen and serum creatinine levels, promoting renal injury and decreases glomerular filtration rate.

Several methods are being employed to prevent or minimize cisplatin-induced nephrotoxicity. However, there is no clinically effective drug to treat this complexity, and thus many natural products such as curcumin, ginseng and pomegranate are being considered due to their anti-inflammatory and antioxidant properties.<sup>114</sup> H Hagar *et al.* experimentally investigated the role of betaine in preventing cisplatin-induced

nephrotoxicity. Betaine as a natural antioxidant substantially improved cisplatin-induced lipid peroxidation and prevented the continuous reduction in antioxidant enzymes. In addition, renal functional and morphological deterioration ineluctably improved upon betaine pre-treatment. A recent study examined cisplatin-induced DNA damage and cell apoptosis through the stimulation of reactive oxygen species. Further, betaine inhibited caspase 3 by receding signal production either *via* intrinsic membrane death receptor proteins such as Fas and TNFR1 or the mitochondrial *cytochrome c* release pathway. Similarly, the protein stabilizing effect of betaine can contribute to protection against cisplatin-induced DNA damage. Hence, betaine supplementation can be effectively used for protection and prevention against anticancer drug-induced nephrotoxicity.<sup>115</sup>

**3.10.3 Betaine relieving cisplatin-induced hepatotoxicity.** The liver performs several important functions in the body such as metabolism of bile acid, cholesterol, plasma protein secretion, hormone metabolism, glucose synthesis and storage, thus maintaining a healthy life. All these pivotal functions are performed by hepatocytes and several other cells in the liver, leading to systematic homeostasis.<sup>116</sup> Mitochondria play a central role in all these biochemical processes. Moreover, hepatic mitochondrial enzymes play a role in lipolysis, one-carbon metabolism, gluconeogenesis and oxidative phosphorylation. Liver toxicity is initiated by several mechanisms such as hepatocellular toxicity and metabolism-related and immunological hepatotoxicity. Furthermore, anticancer drug-induced mitochondrial dysfunction is a challenging aspect in liver injury, which is responsible for enhanced oxidative stress.<sup>117</sup>



Table 3 *In vitro* experiments using gene knockout/overexpression

Cancer type	Cell line/model system	Targeted genes	Experimental approach	Key findings	References
Prostate (DU-145)	DU-145	Bax, caspase-3	Overexpression/knockout	Apoptosis blocked with caspase-3 KO; affirms oxidative-stress-induced apoptosis	28
Prostate (C4-2B)	C4-2B	PI3K, AKT, NF- $\kappa$ B, Bax, caspase-3	Overexpression/knockdown	Overexpression of AKT counteracts apoptosis; upregulated Bax/caspase-3 affirms mechanism	29
Cervical	Hela	p53, cyclin D1, caspase-3	Knockout/overexpression	p53 KO suppresses apoptosis; overexpression of cyclin D1 blocks cell cycle arrest	26
Oral	CAL-27	SIRT1, cyclin B1, caspase-3	siRNA knockdown	SIRT1 knockdown abolishes the betaine effect, which confirms its key role	30
Lung	A549	NF- $\kappa$ B, p38 MAPK	Gene silencing/inhibitor	NF- $\kappa$ B silencing increases effect; p38 inhibition inhibits apoptosis	24
Colon	RAW 264.7 macrophages	NF- $\kappa$ B, IL-6, COX-2, TNF- $\alpha$	Gene knockdown (siRNA), pharmacological inhibition	siRNA NF- $\kappa$ B or IL-6 establishes inflammatory cytokine function in CRC development	27
Liver	HepG2, rat liver cells	p16, c-Myc, caspase-3	Methylation/epigenetic regulation, gene expression modulation	p16 hypermethylation and c-Myc hypomethylation reversed by betaine	65
Breast	MCF-7, BR-75-1, MDA-MB-231	VEGF, TSP-1, caspase-3	Protein expression modulation (transfection, siRNA)	VEGF/TSP-1 decreased after beta-derivative treatments; increase in caspase-3 confirms apoptosis	23
Drug toxicity (cardiac, kidney, and liver)	<i>In vitro</i> & <i>in vivo</i> mouse/rat models	PUMA, TNFR1, SOD, GSH, NF- $\kappa$ B	Gene/protein expression analysis (qPCR, western blot, siRNA)	Betaine overturns Dox-induced upregulation of PUMA/TNFR1; caspase-3 suppression maintains organs	115

Cisplatin is a widely used chemotherapeutic drug, which is being employed for the treatment of various cancers but at the same time is responsible for hepatotoxicity. It rapidly penetrates body tissues by passive transport, increasing its concentration in liver. However, diverse methods are being employed to ameliorate cisplatin-induced hepatotoxicity, for instance, molsidomine (MOL) is a pro-drug and robust vasodilator, and it also exhibits antianginal effects, which can be effectively used against oxidative stress and hepatotoxicity.<sup>118</sup> In addition, various natural antioxidants such as green tea, *Acacia hydaspica*, *Ephedra alata* and *Curcuma longa* have been experimentally investigated to mitigate cisplatin-caused hepatotoxicity.<sup>119</sup> Similarly, betaine is also a natural and organic antioxidant, which can be utilized for analogous outcomes.

H Hagar *et al.* experimentally scrutinized the use of betaine to mitigate cisplatin-induced hepatotoxicity. Previously, the administration of betaine effectively decreased lipid peroxides and augmented the concentration of enzymatic and non-enzymatic antioxidants (SOD and GSH, respectively). This research group reported that the powerful reducing and direct antioxidation effect of betaine can substantially validate its hepatoprotective property. Further, oxidative hepatic injury caused by hepatotoxicants such as chloroform, ethanol and lipopolysaccharides can be prevented by the action of betaine. Moreover, the metabolism of sulfur-containing amino acids and methionine synthesis by betaine can enhance the production of reduced glutathione, which in turn supports the detoxification role of the liver. The expression of an oxidative stress-induced transcription factor (NF- $\kappa$ B) in hepatic tissues by the action of cisplatin was nullified by pre-treatment with betaine. Furthermore, the inhibitory effect of betaine on caspase-3 significantly contributed to the mitigation of hepatic injury (Table 3).

Owing to the anti-inflammatory and antioxidant properties of the non-toxic and stable betaine, its supplementation can alleviate cisplatin-induced hepatotoxicity and act as a hepatoprotective agent during chemotherapy (Fig. 8).<sup>120</sup>

### 3.11 Clinical trials of betaine

Direct clinical trials of betaine are limited; however, other observational and preclinical studies have provided insights into its benefits. In observational studies, the link between cancer risk and betaine level was explored. A cohort of studies presented a meta-analysis demonstrating that the betaine level is linked to a decrease in the risk of cancer cases, more specifically colorectal cancer. The shared relative risk by combining top and bottom levels of betaine was 0.93.<sup>121</sup> Animal studies investigated the effect of betaine on tumor growth. In one study, rats were utilized and there was significant decrease in their tumor volume and weight compared to the control group. However, the linkage of betaine with C-phycoyanin (C-PC) failed to show any effective synergistic effects beyond C-PC alone.<sup>122</sup>

### 3.12 Safety profile and dose limitations of betaine

Betaine has demonstrated potential therapeutic effects in cancer research given that it inhibits the growth of cancer cells by inflammation, oxidative stress and apoptosis. In its dose-

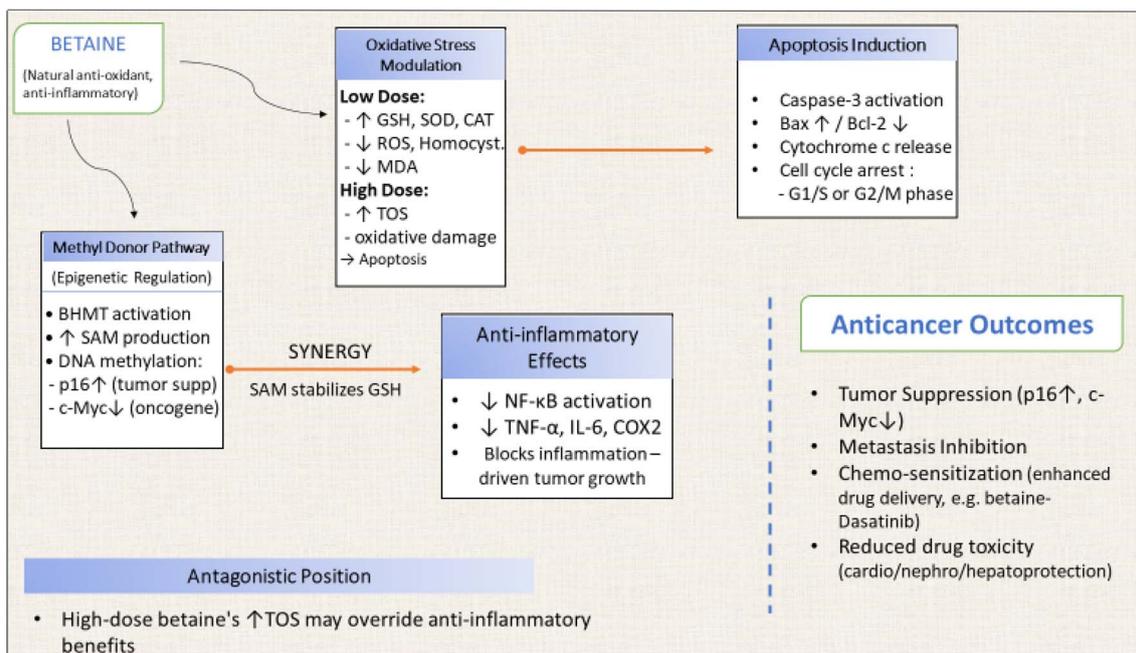


Fig. 8 Anticancer effects of betaine through multiple pathways.

dependent mechanism, betaine behaves as an oxidant at higher concentrations *e.g.*, in human prostate cancer, increasing its concentration from 40 to 50 mg mL<sup>-1</sup> resulted in a decrease in cell viability and increase in malondialdehyde (MDA), pro-inflammatory cytokines including IL-6, TNF- $\alpha$  and total oxidant status (TOS). Owing to these effects, there was reduction in antioxidant defenses such as total oxidant status (TOS), catalase (CAT) and superoxide dismutase (SOD).<sup>123</sup>

In clinic, betaine has been used to target deficiencies in critical methyl donors involved in gene expression regulation and DNA methylation, that is, *S*-adenosylmethionine. In acute lymphoblastic leukemia patients, low SAM levels cause interruptions in chemotherapy, and in this case, betaine in supplement form, *i.e.*, 25 mg kg<sup>-1</sup> twice daily and gradually increasing to 150 mg kg<sup>-1</sup> day<sup>-1</sup>, increased the plasma SAM concentrations by facilitating chemotherapy and alleviating SAM-related metabolic challenges.<sup>124</sup>

In terms of safety, betaine has shown good tolerance in humans, where studies demonstrated no adverse effects with an oral dose 4 g day<sup>-1</sup> in individuals. However, there may be mild side effects with a higher dose such as gastrointestinal discomfort. In animal trials, its low toxicity was further accentuated with lethal doses (LD<sub>50</sub>) in rats in the range of 10–11 g kg<sup>-1</sup> by oral administration.<sup>125</sup>

## 4. Conclusion

Numerous types of cancers have been cured with natural components, many of which are included in everyday diets. They offer considerable defence against numerous malignancies. Antioxidant compounds shield cells from damage,

combating cancer and other disorders. Betaine, the natural substance covered in this review, has anticancer properties. In this study, we discussed the diverse antitumor activities of betaine and its complexes, which can serve as a theoretical basis for their role as a natural defence against cancer. In this review, we evaluated the role of betaine in different types of cancers with different potential mechanisms such as breast cancer, liver cancer, lung cancer, prostate cancer, cervical cancer, and colon cancer. Studies have shown that betaine in combination with anticancer drugs exhibits striking aptitude to augment the chemotherapeutic action of drugs. Furthermore, betaine as a natural compound is quite compatible with living organisms, showing a favourable safety profile and minimal side effects. Thus, the use of betaine in the treatment of cancer is growing quickly with preclinical trials on animals and observations for the suppression of cancer.

## Data availability

No primary research results, software or codes has been included and no new data were generated or analysed as part of this review.

## Conflicts of interest

Authors declare no conflict of interest.

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