

## A review: Therapeutics potentials of phytochemical drugs and their loading in pH specific degradable Nano-drug carrier targeting colorectal cancer

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### Abstract

Increasing incidents of colorectal cancer have shifted researchers' attention to the production and improvement of anti-cancer drugs by the scientific investigation of vast pool of synthetic, biological and natural products. Thymoquinone and thymohydroquinone are considered the ideal compounds for the cancer therapy as they are economically and environmental friendly and have less toxicity level to the survival and diseased model up to increased dosage level. For colorectal cancer, researches are shifting towards the oral drug delivery instead of injection, as administering drugs through oral route shows maximum absorption of drugs, improves patient life quality and is cost-effective. Naturally occurring polysaccharides as oral drug carriers, such as pectin, have the ability to break down completely in colon, making it suitable for targeted drug delivery against cancer cells. Pectin with polymeric base is an efficient nano drug carrier. The current study reviews the delivery of thymoquinone/thymohydroquinone through pectin nano carriers to treat colorectal cancer.

**Keywords:** Colorectal cancer, Thymoquinone, Thymohydroquinone, Pectin, Nano drug carriers, Phytochemical drugs.

### Colorectal Cancer

Colon cancer, also referred to as colorectal cancer, is caused by the growth of cancerous cells in colon, rectum or caecum.<sup>1</sup> In the United States, colorectal cancer is the second largest reported cancer, and ranked fourth among all cancers globally.<sup>2</sup> The threat of emerging sporadic colorectal cancer is significantly moderated by environmental factors and lifestyle attitudes including obesity, physical inactivity, smoking, alcohol consumption and family history.<sup>3</sup> The common pathways involved in the manifestation of colon cancer are the suppression of tumour suppressor or instability in chromosomal pathway which is resulted by mutation of adenomatous polyposis coli (APC), tumour suppressor protein p53, activated telomerase, deletion of SMAD 4 (Mothers against

decapentaplegic homolog 4: A Cell signalling protein) and K-ras genes.<sup>4</sup> Chemotherapeutic drugs are given as combination of leucovorin, 5-fluorouracil and oxaliplatin when colon cancer proceeds to lymph nodes.<sup>2</sup>

However, to improve the quality of cancer treatment, the oral drug delivery of anti-cancer agents in comparison with injection was found to be more effective in terms of maximum absorption of drugs, patient life quality and cost-effectiveness.<sup>2</sup> Colon-targeted oral drug delivery, without being observed in upper gastro intestinal tract, can increase drug bioavailability at target site, possibly allowing minimum absorption in system resulting in the reduction of the administered dose and decrease of systemic side effects.<sup>5</sup> Colon-targeted delivery by pro-drugs, potential of hydrogen (pH), azopolymers, time and pressure-sensitive methods have respective pros and cons. Each of them has limitations in site specificity, ease of preparation, toxicity and reproducibility of performance.<sup>2</sup> Numerous factors effect the colon drug delivery system, natural polysaccharides as a drug carrier can overcome the issues of toxicity, bioavailability and safety of drugs.<sup>6</sup> The polysaccharides as a drug carrier has the ability to hydrate and swell while passing through GIT and releases drug at the targeted site in the colon in presence of colonic bacteria and enzymes.<sup>7</sup>

### Phyto-Chemical Compounds

The usages of plant-derived bioactive components are increasing again in the medicinal field, especially against cancer.<sup>8</sup> In cancer treatment, nanotechnologies are found to improve bioavailability, solubility and specific targeting while reducing the doses, toxicity and achieving steady-state curative levels<sup>9</sup> (Figure-1).<sup>10</sup> Due to wide concentration of scientist in oncology, advancements are progressing towards nanotechnology, which combine nano drugs and cancer.<sup>11</sup> Expected drawbacks of traditional chemotherapeutic agents, scientists focused to develop molecular targeted treatments involving nanotechnologies which combines nano drugs and cancer.<sup>11</sup> This nano drug delivery system has potential for enhancing drug concentration and efficiency in cancer cells while avoiding any toxic effects to healthy cells.<sup>9,11</sup>

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The role of chemotherapeutic drugs results in apoptosis in cancer cells and the comprehensible drugs include doxorubicin (DOX), 5-fluorouracil, paclitaxel, epirubicin, oxaliplatin, cisplatin, etc.<sup>10</sup>

Varieties of phytochemicals components are present in a single plant and the fact is that same phytochemical can exist in more than one plant.<sup>12</sup> Most of the anti-cancer activities of phytochemicals involve inhibition of proliferation of cells, angiogenesis, reserve mitosis, destruction of inflammatory process involving cyclo oxygenase-2 expression and initiation of apoptosis at different stages of different cancers.<sup>13</sup> Zheng et al. noted the cytotoxicity of triptolide and triptolide loaded polymeric micelles against HT29 human adenocarcinoma cells.<sup>14</sup> Triptolide is extracted from the Chinese herb having anti-cancer activities *Tripterygium wilfordii*. For synthesis of loaded polymeric micelles (TP-PM) methoxy poly (ethylene glycol)-poly lactic acid (MePEG-PLA) copolymer solvent evaporation method was used.

Both the free triptolide and the TP-PM had a dose- and time-dependent effect on the HT-29 cells, however, the inhibitory effects of TP-PM on the tumour cell growth were more significant for all incubation times and concentrations. Hence, the polymeric micelles served as an excellent carrier of TP and reduced its toxicities.<sup>14</sup>

Chemotherapeutic effects of phytochemical polyphenol compound honokiol were studied by Dong et al. They loaded drug into the bio degradable star-shaped micelles

**Table:** Types of phytochemicals and pharmaceutical drugs with different type drug carriers against Colorectal cancer.

Type of drugs	Type of drug carriers	References	
Phyto Chemical drugs	Triptolide	MePEG-PLA copolymer micelle	14
	Honokiol	MPEG-PCL star shaped micelle	15
	Luteolin	MPEG-PCL micelle	16
	Thymoquinone	PLGA nano particles	17
Pharmaceutical drugs	Quercetin	Zein-Pectin nano drug carriers	64
	5-Fluorouracil	Eudragit S 100 citrus pectin nano particle	65

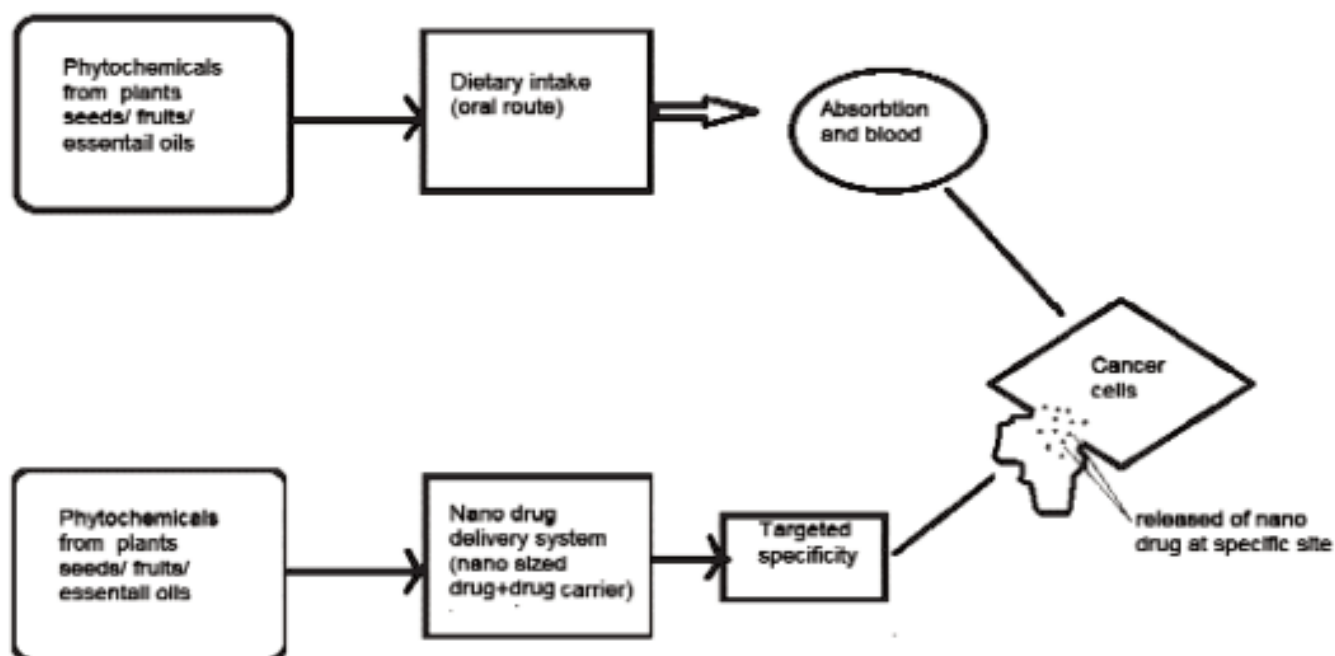
MePEG-PLA: Methoxy poly(ethylene glycol)-poly lactic acid

MPEG-PCL: Monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -aprolactone)

PLGA: Poly-lactide-coglycolide.

monomethoxy poly (ethylene glycol) (MPEG) and poly ( $\xi$ -caprolactone) (PCL) by ultrasonication. The average particle size of obtained honokiol micelle was about 40nm and was treated to CT26 murine colon carcinoma cells. They showed anti-proliferative effect against the CT26 cells in a dose dependent fashion.<sup>15</sup>

Luteolin (Lu) is a flavonoid with anticancer activity loaded in monomethoxy poly (ethylene glycol)-poly ( $\epsilon$ -aprolactone) (MPEG-PCL) micelles in vivo to evaluate C-26 colon carcinoma cells by self-assembly method. The pharmacokinetics of free luteolin and Lu/MPEG-PCL micelles was studied in rats; it was found that the bioavailable concentration of luteolin was more when the Lu/MPEG-PCL micelles were used. Moreover, the



**Figure-1:** Effective Mechanism of Nano drug system as compare to dietary intake of phytochemical compounds.<sup>10</sup>

Lu/MPEG-PCL micelles inhibited the growth of C-26 colon carcinoma cells at IC<sub>50</sub> of  $12.62 \pm 2.17 \mu\text{g/mL}$ . Hence, the study recommends that the encapsulation of Lu into MPEG-PCL micelles created an aqueous formulation of Lu with potential anticancer effect, increasing efficiency up to 95.6%.<sup>16</sup> Ravindran et al. studied anti-proliferative activities of thymoquinone loaded in poly (lactide-co-glycolide) (TQ-PLGA) nanoparticles using human colon cancer HCT 116. The TQ-PLGA nanoparticle had encapsulation efficiency around 94% and ranged between 150 and 200 nm in size. Apart from this the nanoparticles were active in inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and in suppressing the expression of cyclin D1, matrix metalloproteinase (MMP)-9, vascular endothelial growth factor (VEGF) when compared to the free thymoquinone. On the whole, the results demonstrate that encapsulation of TQ into nanoparticles enhances its anti-proliferative effects<sup>17</sup> (Table).

There has been a continued search for a new anticancer drug and a better method of administration. Natural compounds are found to suppress the growth of cancer cells by inducing programmed cell death which is indicated by the notable changes such as deoxyribonucleic acid (DNA) damage, increase in reactive oxygen species (ROS) generation,<sup>18</sup> release of cytochrome C,<sup>19</sup> activation of caspases, cell cycle arrest,<sup>20</sup> activation of NF- $\kappa$ B<sup>21</sup> and down regulation of MMP, Bax (BCL2-Associated X Protein), cyclin D and VEGF, along with visible morphological apoptotic changes.<sup>22</sup>

However, there are enormous studies showing the anticancer property of bioactive compounds of plants but this review particularly deals with the nanotechnology-based drug delivery of TQ and thymohydroquinone (THQ) with the help of biodegradable citrus fruit-based polysaccharide pectin drug carrier.

TQ and THQ are the quinones existing in many plant species like *Monarda didyma* L., *Monarda media* Willd., *Monarda menthifolia* Graham, *Satureja hortensis* L., *Satureja montana* L., *Thymus pulegioides* L., *Thymus serpyllum* L., *Thymus vulgaris* L. and *Nigella sativa*.<sup>22</sup> However, the occurrence ratio of both components may vary with the species and also on the other environmental factors like temperature, pH and laboratory extraction methods.<sup>10</sup> For the particular review *N. sativa* and *T. vulgaris* plants are considered due to their wide distribution worldwide and extensive use in dietary as spice in many cuisines.<sup>23</sup>

### **Nigella Sativa**

*Nigella sativa* (*N. sativa*) (family: Ranunculaceae) is annual

flowering plant, emerging as a marvel having wide spectrum of pharmacological potential. *N. sativa* is commonly known as black seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia, and it is cultivated in many countries in the Middle Eastern Mediterranean region, Southern Europe, and in India, Pakistan, Syria, Turkey and Saudi Arabia.<sup>24</sup>

*N. sativa* has been widely studied for its natural and therapeutic activities. It has potent potential against cancer, hypertension, diabetes, analgesia and inflammation, and has microbial, hepatic, gastrointestinal and many other antioxidant properties.<sup>25</sup> It is stated in many studies that the therapeutic properties of this plant are mainly due to the presence of most important phyto-compound, TQ, which is a main bioactive component of the essential oil of seed.<sup>26</sup>

### **Thymus Vulgaris**

*Thymus vulgaris* (*T. vulgaris*) is a flowering plant of mint family Lamiaceae. It is native to Southern Europe and South East Asia and can be cultivated in many countries of the Mediterranean region.<sup>27</sup> Thyme is major bioactive component of the essential oil of dried herb along with other biochemical compounds including TQ and THQ which shows a wide range of biological and medicinal properties such as antiseptic, expectorant, antispasmodic, anthelmintic, anti-inflammatory, antioxidants and lately as anti-cancer agents.<sup>28</sup> Thyme extraction from thymus species as an essential oil, containing thymol as a main compound, can be easily converted to oil with TQ and THQ as the main component. With the appearance of even small quantities of these compounds, the thyme essential oil becomes a more potent anticancer and antioxidant.<sup>29</sup>

### **TQ and THQ**

Thymoquinone (2-isopropyl-5-methyl-benzoquinone) is a phytochemical component showing potent anti-cancer activities against different tumour models.<sup>30</sup> It has proved to be effective against several types of cancer cell lines in which the classical hallmark of apoptosis such as chromatin condensation, translocation of phosphatidyl serine across the plasma membrane, and DNA fragmentation have been documented in TQ-treated cells.<sup>31</sup> There is an increasing research interest in TQ to evaluate its anticancer activity against different types of cancer.<sup>32</sup> Abu Khader investigated new findings, suggesting new mechanisms of anticancer activity of TQ against breast cancer in vitro and in vivo models.<sup>33</sup>

Thymohydroquinone (2-methyl-5-isopropylhydroquinone) is also the major component along with the TQ. The

present knowledge about antitumour activity of THQ is very limited and till today there is no data *in vivo*.<sup>34</sup> THQ, due to its less stable nature than TQ, may break earlier and its oxidation results in the formation of more TQ. Both can be extracted from the same plants but in different amounts.<sup>22</sup>

### Anti-cancer mechanism of TQ and THQ

The assumed mechanism of TQ action involves manifold paths which play significant roles in cancer development. It was reported that TQ prompts intrinsic pathways of apoptosis through the activation of caspases cascade. The activation of caspase-8 highlights the effect of TQ on Bcl2 and the role of mitochondria in thymoquinone-induced apoptosis in human squamous cell carcinoma, in human osteosarcoma p53-null MG63 cells and in p53-null HL-60 myeloblastic leukaemia.<sup>35</sup> The process of apoptosis in human breast cancer cell line (Michigan Cancer Foundation-7) MCF7/DOX cells was also found to be mediated through a caspase-dependent manner which triggered the intrinsic pathway through the activation of caspase-3, -7, -9 and the cleavage of poly(ADP-ribose) polymerase (PARP) but not caspase-8.<sup>36</sup> An increase of TP53 expression level in MCF7/DOX cells indicated the p53-dependent apoptosis after treatment with TQ resulting in the reduction of Bcl2 protein and reduction in the Bcl2/Bax ratio.<sup>36</sup> Studies showed anticancer effect of TQ on different type of cancer cells through *in vitro* and *in vivo* which indicate the involvement of TQ in different cell death signalling pathways including apoptosis, proliferation, angiogenesis and tumour-induced immunosuppression.<sup>37</sup> Every type of cell can secrete transforming growth factor which is dependent on the cell response to the transforming growth factor (TGF- $\beta$ ) receptors. Increase or decrease in their functions and its downstream pathway can lead to cancer. So far, the anticancer mechanism of TQ is not fully understood; however, several modes of action have been described depending on the stimulus and the cellular context.<sup>38</sup>

A study revealed that TQ reduced the cell viability and convinced apoptosis in human colorectal cancer cell line HCT116.<sup>39</sup> Products of TQ were tested in HL-60 cells and 518A2 melanoma by Effenberger et al., who reported that TQ has anti-proliferative effects in HL-60 cells, 518A2 melanoma blood cancer, in MCF-7 breast carcinoma and in HCT116 colon cancer. They concluded that the derivatives of TQ induce apoptosis associated with DNA laddering, a slight increase in reactive oxygen species and cell death.<sup>40</sup> Woo et al. in their study showed that TQ induced cell death, i.e. apoptosis, and reduced proliferation in xeno-grafted mouse model effected with breast cancer.<sup>41</sup> El-baba et al. assessed the TQ ability of

inducing apoptosis by conformational changes in the colorectal cancer targeting PAK1 (protein activated kinase).<sup>42</sup> Many investigations are made on the anti-proliferative, anti-inflammatory and anti-cancer effects of extracted TQ in recent years against cancers like breast, renal cancer, colorectal, prostate and cervical cancers by many investigators.<sup>43</sup>

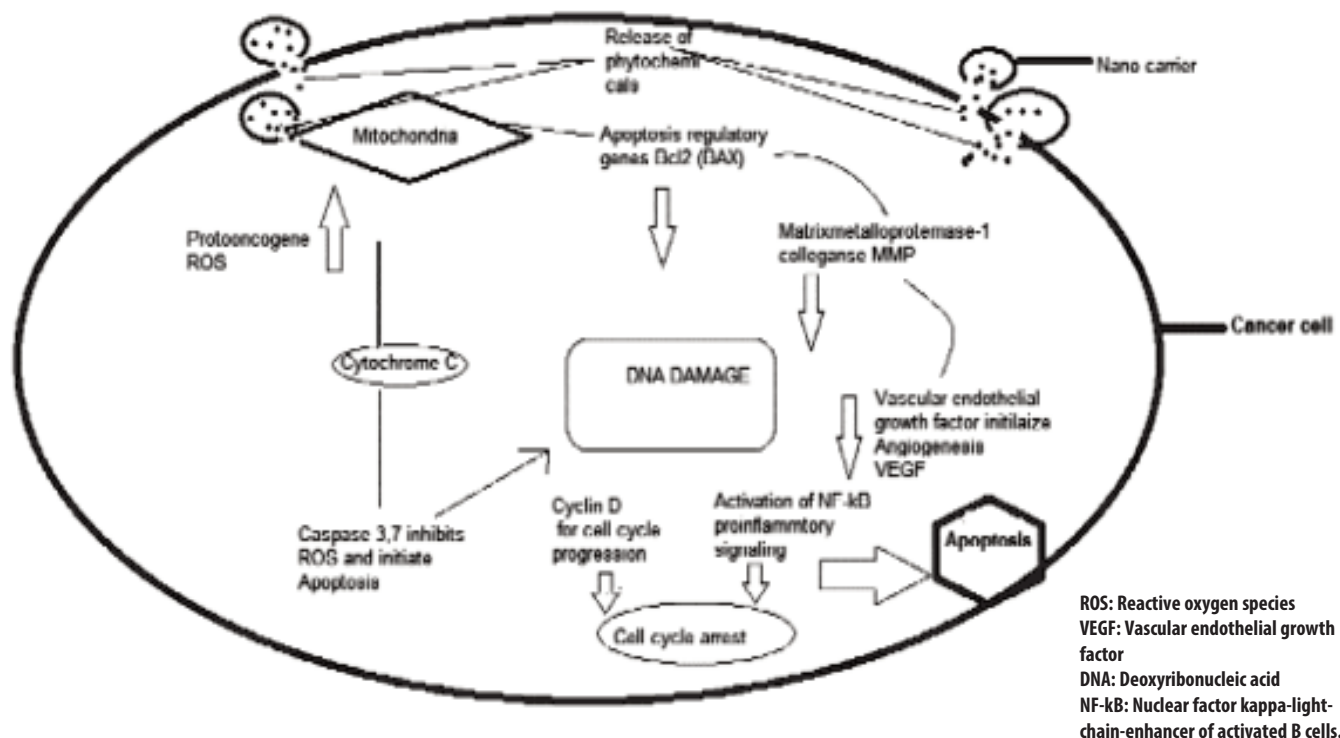
Ravindarin et al. and Nallamuthu et al. performed research on TQ chemo-sensitisation potential on employing polymer-based nanoparticle approach to improve upon its bioavailability and effectiveness against cancer cells including colorectal cancer. They concluded that encapsulation of TQ into nanoparticles enhances its anti-inflammatory, anti-proliferative, and chemo-sensitising properties. THQ studies on such basis are not being reported yet.<sup>17,44</sup>

Khan et al. and Ravindarin et al. emphasised more investigation regarding anti-cancer and anti-inflammatory activities of TQ. More research work is needed on TQ and THQ extraction from some important medicinal plants because it is a safe and promising anticancer component.<sup>45,17</sup> Also, the exact molecular mechanisms of TQ and other components like THQ, which are considered in very few studies, on different cancers should be investigated with more emphasis because current understandings are mostly uncertain. Like other phytochemical compounds, loading TQ and THQ in nano drug carrier shows potent target specificity and better efficiency.<sup>17</sup>

### Nano Drug Delivery

Scientists are focusing on the anti-cancer activities of phytochemicals leading to the delivery of these drugs to specific sites via nano drug carriers, sized in nanometres.<sup>46</sup> The nano system having nano-materials may vary from size of 1-100nm carrying drug to the specific site by following the techniques of nanotechnology.<sup>47</sup> Nano drug carriers carry these drugs to the targeted destination without leaking or destroying them before they reach to the final destination.<sup>48</sup> The nano-sized drug carriers having controlled shape, size, chemistry and surface charges carry drugs to the specific sites and enhance their functions by up to 40 times.<sup>49</sup>

For cancer drug delivery first step involves leaking of nano medicine out of the blood stream into the affected blood vessels.<sup>50</sup> These nanoparticles have overcome the problem of estimation of right doses of the drug to the affected area by keeping the drug away from healthy tissues/cells and delivering to the targeted site.<sup>51</sup> Different types of nano drug carriers are used to deliver drug in cancer therapy. This is because these nano carriers effortlessly target the cancer cells by fabricating them



**Figure-2:** Molecular mechanism of nano drug loaded in nano drug carrier to the specific target (Cancer cell).<sup>52</sup>

from normal cells (Figure-2).<sup>52</sup>

Delivery of drugs to the target sites, loaded in nano drug carrier, depends on the small size and site-specificity. The four kinds of focusing characteristics of nano drug carriers are active, passive, temperature and pH sensitivity.<sup>53</sup>

Subranabiana et al. in their review concluded that for nano drug delivery, micelle with polymeric base is ideal nano carrier. For micelle the common polymeric bases considered are poly-lactide-co-glycolide (PLGA), poly (ethylene glycol) (PEG), poly lactic-acid (PLA) and methoxy poly (ethylene glycol) (mPEG) due to their good biocompatible and biodegradable behaviour.<sup>10</sup> The nano-drug delivery of phytochemicals has been extensively investigated, still further investigations are needed to find outcome of the combination of nano-drug delivery and carrier system for cancer. Furthermore, clinical studies on nano-drug delivery as well as the mode of administration should be carried out to encourage them in the field of medicinal oncology.<sup>48</sup>

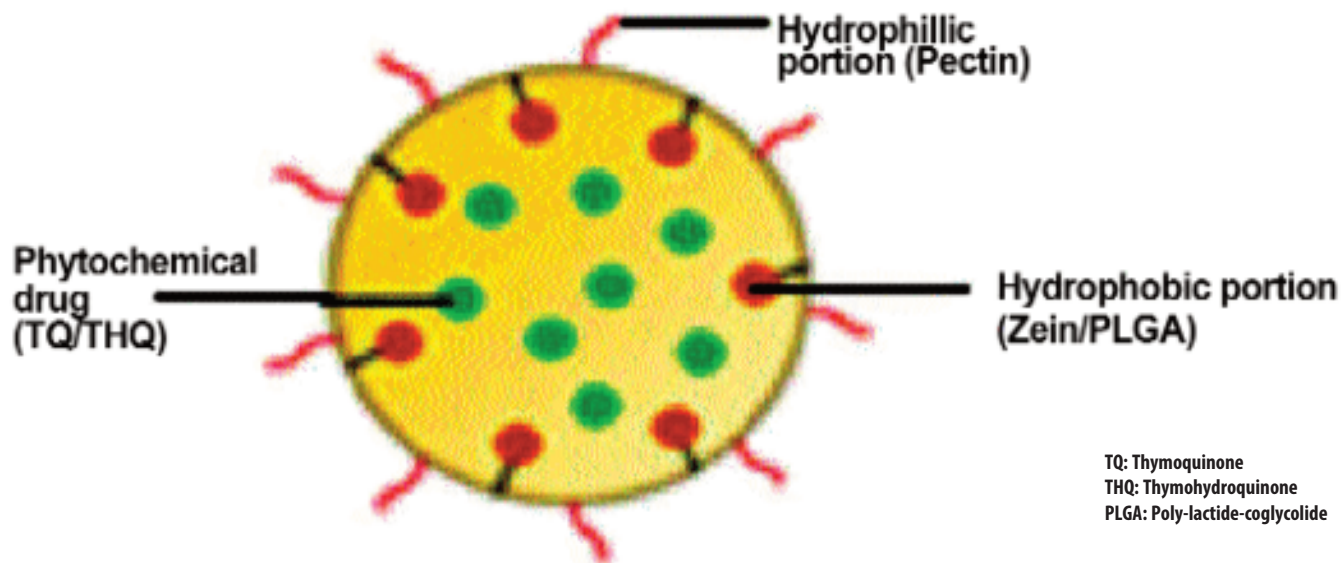
### Pectin Colon-Specific Drug Carrier

Pectin is a natural polysaccharide predominantly found in the cell wall of terrestrial plants.<sup>54</sup> It has 1,4 linked  $\beta$ -D-galactosyluronic acid residues, different neutral sugars such as rhamnose, galactose and arabinose, and amounts of other non-sugar components like acetic acid,

methanol, phenolic acids and amide groups.<sup>55</sup>

Due to the complete fermentation of pectin in colon, it is suitable for use as colon-specific drug delivery carrier in treatment of colon rectal cancer and other colon diseases. Pectin in colon is digested in the form of short-chain fatty acid that regularise the micro-flora in gut by regulating apoptosis in colonic crypts, enhancing the growth of colocyte crypts, preventing pathogen overgrowth, lowering pH and affecting galectin network.<sup>56</sup> Pectin in colon cancer can inhibit mutation and expressions of galectin-3 biological functions. Improved citrus pectin with high degree of esterification and molecular weight increases the bioactivity and defensive roles against colon cancer in mouse model by inhibiting galectin-3 in mouse model.<sup>57</sup>

In inventing colon-specific drug delivery systems, numerous preparation approaches have been taken in the past few years to prevent pectin-based matrix from undergoing early drug release in the upper GIT. Pectin, in combination with a cross-linking agent or a polymer, may also be employed itself as a delayed release coat to be applied onto a drug core via film or compression coating technique.<sup>2</sup> Pectin has excessive ability to dissolve in water creating problem colon targeted drug delivery which can be overcome by preparing pro-drugs which will release the free drug upon arrival at colon. Coating of hydrophobic



**Figure-3:** Proposed Model Nano drug delivery system of Pectin micelle matrix loaded with phytochemicals (TQ/THQ).

polymer on pectin oral drug delivery system makes it unaffected in upper GIT which upon digestion of pectin releases drug from developed pore.<sup>58</sup> Advancement in oral drug delivery as pectin drug carrier made by complex coat made up of water-soluble polymers, the action of pectinolytic enzymes on the coat complex results in leaching of pectin and drug release in colon.<sup>59</sup>

The hydrophilic polymer counterpart of pectin, once released from complex, similarly becomes freely solvated, swells and leads to distortions in coat, thereby further facilitating drug release. Enzymatic degradation of pectin components forms the primary mechanism of colon-specific drug release. In vivo assessments indicate that colon-specific pectin-based oral dosage forms can be designed primarily through coating of drug matrix with pectin-ethylcellulose or Eudragit film having superdisintegrant Explotab® V17.<sup>60</sup> It is imagined that the reproducibility of matrix digestion and drug release at colon is an interaction effect of colonic pH and microflora environment with the physicochemical properties of drug, pectin and other excipients of the dosage form.<sup>2</sup> The pectin-derived oral drug carriers are being extensively used in delayed release dosage system in colonic cancer. Gelatin, alginate and xyloglucan are some polymers explored in formulation of pectin-based drug delivery system resulting in different drug delivery formulation like beads, pellets, film and upto micro and nano scale structures.<sup>61</sup> Pectin receives an overwhelming attention on its practical applications and Implications in drug delivery (Table). However, the pectin has complex structure and physicochemical properties which may vary

with its manufacturing source, extraction temperature, pH and extraction acid type.<sup>62</sup>

Future studies on pectin drug delivery may characterise the physicochemical qualities of pectin and evaluate its structure-activity relationship with orientation to colon-specific drug delivery.<sup>2</sup> The option of pectin-based colon-specific dose form to achieve solely and precisely at a cancer site of cancerous colon is an appealing challenge for pharmaceuticals. Butte et al. explored combination of Pectin with other polymer Eudragit S100. This study revealed that both these polymers have the ability to protect the core in the upper GIT and helps in attaining targeted release of curcumin in the colon. The combination of pectin with other hydrophobic polymers, like PLGA, requires further investigation on their potential use as synchronised drug carrier and chemotherapeutic agent through a multi-disciplinary approach.<sup>63</sup>

Danhya et al. successfully developed zein-pectin nano drug carriers which were biodegradable, non-toxic nanoparticles, made solely from natural polymers. Zein-pectin nanoparticle comprises a hydrophobic zein core and a hydrophilic pectin core, loaded with model drug quercetin.<sup>64</sup> Subudhi et al. developed effective Citrus pectin Nanoparticles coated on Eudragit S100 (E-CPNs) for the colon targeting drug 5-Fluorouracil (5-FU). This combination of drug carrier effectively guarded nanoparticles upto the colonic region where these nanoparticles released drug for a prolonged period of time.<sup>65</sup>

A novel nano system having pectin with polymeric-based PLGA loaded with phytochemical drugs (TQ/THQ) to the targeted site (colon) for studying their therapeutics potentials and efficiency rate against colorectal cancer is proposed for future study (Figure-3).

## Conclusion

Phytochemicals TQ and THQ individually would be potent anti-cancer agents for different cancers, particularly for colorectal cancer, when loaded in pH-specific drug carriers. This review also concluded that pectin with polymeric base could be an efficient drug carrier up to the nano level for oral delivery.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** None.

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