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Review Article

Anti- Cancer Activity of Thymoquinone in Gasterointestinal Tract: A Comprehensive Review

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ABSTRACT

Gastrointestinal (GI) cancers are known as malignant condition of the GI system associated with the organs which have role in digestion. More cancers and more deaths from cancer are related to the cancers occurred in this system. *Nigella sativa (N. sativa)* seeds which are traditionally used as a food additive, preservative or a spice in many cultures, has been considered for treatment of various diseases. Thymoquinone (TQ) is the main bioactive component of the volatile oil of *N. sativa* seeds. Therapeutic effects of TQ for treatment of various diseases such as cancers, in both *in vivo* and *in vitro* conditions, have recently been interested. In this comprehensive review, we summarized the new studies related to anti- cancer activity of TQ associated with its possible mechanisms in GI cancers including oral cancer, esoghageal cancer, gastric cancer, colorectal cancer and also liver and pancreatic cancers. It is concluded that TQ could be considered as an anticancer agent alone or in combination with chemotherapeutic drugs in treatment of GI tract cancers, however well designed clinical trials in humans are required to confirm these effects.

Keywords: Thymoquinone, Ggasterointestinal Cancer, Hepatic Cancer, Pancreatic Cancer

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Gastrointestinal (GI) cancers are known as malignant situations of the GI system and related organs with digestion including the esophagus, stomach, liver, pancreas, small intestine, colon and rectum which are responsible for more cancers and more deaths from cancer than any other system in the body (1, 2). Use of traditional medicine for cancer treatment is usually free of side effects and generally inexpensive (3). *Nigella sativa (N. sativa)* as a herbal plant of the *Ranunculaceae* family has a long-term history in different medicinal and nutritional goals and is growing in Southwest Asia,

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southern Europe and North Africa (4). The seed is named black seed or black cumin in English. Black seed consists of over 100 valuable components. It is a rich source of carbohydrates, proteins, essential fatty acids, minerals and vitamins (5-7). Thymoquinone (TQ) as the main component of volatile oil extracts of *N. sativa*, has recently been considered for its antioxidant, immunoprotective and antitumor features (8-11). Using of a combination of dietary phytochemicals with different mechanisms has been explained as a favorable approach to monitoring of various cancers with side effects. It has been reported some effects of TQ on cancer cells contains DNA injury, cell cycle arrest, reactive oxygen species (ROS) dependent apoptosis and decreased cell proliferation (10, 12). The present review provides the recent studies from 2004 to 2018 that regarding the anti cancer activity and related mechanisms of TQ in GI cancers.

Methods

Pubmed, google scholar and science direct databases were searched from 2004 until 2018. The keywords used were the following terms: *thymoquinone,* gasterointestinal cancer, hepatic cancer and pancreatic cancer. Searching was done on articles with English language.

Results

Oral and esophageal cancer

Oral cancer is an usual tumor which can be accompanied with high morbidity and death. P38 mitogen-activated protein kinase (MAPK) has role in cell proliferation and differentiation. The study was done by Abdelfadil et al. showed that thymoquinone can cause to apoptosis in oral cancer cells through downregulation of p38β MAPK (13). Hu et al. explored the chemosensitive property and

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mechanism of TQ in increasing effect of cisplatin (DDP)-induced apoptosis of esophageal carcinoma (EC) both *in vitro* and *in vivo*. Based on the *in vivo* and *in vitro* results, TQ enhanced chemosensitivity and cellular apoptosis of DDP via blocking the activation of Janus kinas 2/ Signal transducer and activator of transcription 3 (JAK2/STAT3) way. According to this study, TQ with DDP may interfere the development of EC and can be considered as a chemotherapeutic agent in EC (14).

Gastric cancer

Gastric cancer is the third causing of death related to cancer worldwide. In spite of reducing in its occurrence in some areas of the world, this cancer is still considered as a main clinical challenge related to delay in diagnosis with a weak prognosis and little treatment choices (15). 5-fluorouracil (5-FU) is the most common chemotherapeutic agent in stomach cancer alone or composed with other cytotoxic drugs (16, 17).

Lei et al. for the first time investigated the chemosensitizing property of TQ and 5-FU on gastric cancer cells *in vitro* and *in vivo*. The results showed that pretreatment with TQ importantly elevated the apoptotic features in gastric cancer cell lines induced by 5-FU in *vitro.* Furthermore, its found that TQ increased the 5-FU-induced death of gastric cancer cells through decreasing of B-cell lymphoma 2 (Bcl-2) as the anti-apoptotic protein, the increasing of bax as the pro-apoptotic protein, and caspase-3 and caspase-9 activation. Furthermore, combined treatment of TQ with 5-FU showed an importantly more effective antitumor effect than each of them alone in a xenograft tumor mouse model. This study suggested that the TQ/5-FU combined treatment causes apoptosis through increasing of caspase-3 and caspase-9 activation in gastric cancer cells (18).

To find the mechanism of apoptosis induce by TQ in human gastric cancer cells *in vitro* and *in vivo*, the cultured HGC27, BGC823, and SGC7901 cells treated with TQ (0, 10, 25, 50, 75, 100, 125 μmol/ L) for 12 h, 24 h, and 36 h. TQ caused to apoptotic cell death in HGC27 cells in dose dependent manner. TQ prevented the STAT3 but not STAT5 phosphorylation. In addition, TQ decreased the STAT3-regulated genes expression including

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cyclin D, survivin, Bcl-2, and VEGF, and activated caspase-3,7,9. Similar to the *in vitro* results, TQ was greatly effective as an antitumor agent in a xenograft tumor in mice model (19).

Colorectal cancer

Colorectal cancer (CRC) is one of the most usual cancers with high mortality rate. This cancer can cause to 0.5 million deaths each year. To fight this type of cancer, the increase of novel particular drug candidates is required. Non sensitivity and narrow therapeutic properties of its common chemotherapeutic drug show a great problem in CRC treatment (20, 21). 5-FU, as a gold chemotherapeutic agent for the treatment of colon cancer, has capability to invade both healthy and cancerous cells, therefore its use can be associated with side effects (22).

In this context, Kensara et al. studied on whether TQ may increase chemopreventive feature of 5-FU to eliminate the early stages of CRC and explained its mechanism in colorectal tumorigenesis induced by azoxymethane (AOM) in rat. Greatly, 5-FU/TQ compound therapy caused to an more important decrease colorectal tumors induced by AOM and large improper crypts foci than individual drugs therapy. Mechanistically, TQ and 5-FU significantly collaborated to suppress the expression of procancerous wingless integration (Wnt), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), β-catenin, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX-2), VEGF and thiobarbituric acid reactive substances (TBRAS) and increase anti-tumorigenesis transforming growth factor beta (TGF-β1), TGF-βRII, Dickkopf-1 (DKK-1), CDNK-1A, glutathione peroxidase (GPx) and Smad4 expression. This study was the first report which explained the *in vivo* increase feature of composed 5-FU and TQ against early stages of CRC (23).

The other study was done to survey the effect of preserved drug release of 5-FU, TQ and Epigallocatechin-3-gallate (EGCG) on the metabolic action and also morphological alternations in the SW-626 human colon cancer cell line in culture. The findings showed that

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preserved drug release of TQ and EGCG had marked cellular damages and intervention of cellular metabolic actions. Also, glutathione, malondialdehyde (MDA) and nitric oxide showed important changes at 24 hours. Structurally, cellular alternations happened following exposure to TQ and EGCG as early as 24 hours that were similar to cells exposed to 5-FU, too. According to this study, using of natural compound such as TQ can be offer for treatment of colon cancer (24).

In the other study, therapeutic effect of TQ on two different mice colon cancer, was studied. In addition, it investigated the effect of TQ on the C26 mouse colorectal carcinoma spheroids development and evaluated tumor invasion *in vitro*. Intraperitoneally injection of TQ, importantly decreased the sizes and numbers of aberrant crypt foci (ACF) and also tumor multiplicity. Additionally, this study showed that sub-cyto-toxic doses of TQ (40μM) reduced C26 cell invasion and repressed 3-dimensional spheroids growth. Morphologically, apoptotic figures were importantly raised in spheroids treated with TQ (25).

Another investigation showed that TQ caused to apoptosis in human colon cancer HCT116 cells by inactivation of STAT3 through stopping JAK2-and Src□mediated phosphorylation of EGF receptor tyrosine kinase (26).

Zhang and Colleague, investigated features and molecular activity of TQ on colon cancer cells. Findings indicated that TQ treatment importantly reduced cell viability in HCT116 and COLO205 cells, in a dose dependent way. In addition, sensitivity of HCT116 and COLO205 cells to cisplatin treatment was increased by TQ treatment in a concentration-dependent way. Determination of the phosphorylated p65 and NF-κB-regulated gene products, c-Myc and Bcl-2 and vascular endothelial growth factor (VEGF) was done by western blot analysis. The findings showed that TQ treatment importantly reduced phosphorylated p65 level in the nucleus, that exhibited the prevention of NF-Kb action by TQ. TQ treatment reduced c-Myc, Bcl-2 and VEGF levels expression. Also, prevention of NF-κB activity with particular preventer, powered the induction of cell death and resulted to a chemosensitization property of TQ in colon cancer cells. According to this study, TQ could induce cell death and chemosensitized colon cancer cells through prevention of NF-κB signaling (27).

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Topotecan as topoisomerase I inhibitor, is an anticancer drug which is widely applied in the treatment of ovarian, lung, breast and colorectal adenocarcinoma. Because of myelosuppressive feature of topotecan which is related to its primary dose-limiting toxicity, there are research to recognize other chemotherapeutic compounds which can synergistically act with topotecan to enhance its effect and decrease its toxicity. Some studies have shown that cells which are exposed to natural agents for example TQ, followed by oxaliplatin or gemcitabine caused to higher growth prevention in contrast oxaliplatin or gemcitabine treatment alone. Khalife et al. investigated mechanism of action of topotecan in the viability and apoptotic ways in human colon cancer cell lines in contrast to TQ, to find the antiproliferative and proapoptotic properties of TQ on the effect of the topotecan as a chemotherapeutic agent, and to study the powered synergistic property of TQ with topotecan. The results showed that both drugs caused to apoptosis by a p53-independent pathway but the p21 expression was only occurred in treatment with TQ. Stop in cell cycle in the S phase was found with each matter separately, whereas combined treatment caused to enhance in fragmented DNA production. Induce in apoptosis through the extrinsic way was done by both compounds after 24 hours; whereas, after 48 hours, activation of the intrinsic pathway was seen by treatment with topotecan only. According to this study, TQ enhanced the effectiveness of topotecan as a chemotherapeutic reagent through preventing of proliferation and decreasing toxicity through p53- and Bax/Bcl2-independent mechanisms (28).

COX-2 as an enzyme, has a role to the transform of arachidonic acid into PGE2 which can increase expression of COX-2 protein and tumor cell multiplication. The study by Hsu et al. showed that TQ (20 μmol/L) importantly decreased human LoVo colon cancer cell proliferation and migration through decreasing prostaglandin E2 induced COX-2 activation (29).

Other study surveyed pre- and post- treatment of TQ on oxidative stress induced by 1,2-dimethyl-hydrazine (DMH) during beginning and development of colon carcinogenesis in rats. Pre-treatment with TQ (5mg/kg) ameliorated entirely oxidative stress induced by DMH at beginning and established histological alternations and tumor promotion. Additionally, it abolished oxidative stress aggravation at beginning, and importantly decreased tumour occurrence. In contrast, post-treatment with TQ (5mg/kg) restored oxidative status and reduced tumour promotion at beginning. It decreased MDA and antioxidant content at promotion slightly with a little decrease in tumour status and dysplasia level. This investigation showed effectiveness of TQ in protecting and treatment DMH-induced beginning phase of colon cancer and also protective role at promotion. This study supported the idea that TQ may be as a

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chemo-preventive agent in colorectal cancer (30).

Occurrence of colorectal cancer in people with familial adenomatous polyposis (FAP) is high. In the study by Lang et al. chemopreventive features of TQ on FAP were evaluated in mice. TQ treatment (375 mg/kg but not 37.5 mg/kg) reduced large polyp's numbers in the small intestine of mice. TQ caused to apoptosis in the neoplastic mucosa but not in the normal tissue. In addition, treatment with TQ, retained β-catenin at the membrane and reduced c-myc in the nucleus that was accompanied with a decreased cell multiplication in the villi. Also, activation of GSK-3β which induced membranous localization of β-catenin and decreased expression of nuclear c-myc was done by TQ treatment. This study showed that TQ interfered with polyp development in mice by induction of specific apoptosis of tumor-cell and through modulating Wnt signaling by GSK-3β activation and according to these results, TQ might be effective agent as nutritional complement to surgery and chemoprevention in FAP (31).

Recently investigations have showed the anticancer properties of Vitamin D3 (Vit.D) and TQ. Evaluation of synergistic potential of TQ and Vit.D in the control of colon cancer development which was induced by azoxymethane in rat model, was done. The results indicated that combination of TQ and Vit.D (4 weeks before to induction and continued for a total of 20 week) had an important anti- tumor property in contrast to each of them lonely which was showed by great decrease of the grown tumors numbers and major improper crypts foci. In addition, gene expression and/or protein quantification findings showed a great reduction of pro-cancerous molecules (β-catenin, NF-κB, iNOS, Wnt, VEGF, COX-2 and heat shock protein 90 (HSP-90) and also a great increase of anti-tumorigenesis biomarkers (DKK-1, TGF-β1, smad4, CDNK-1A and TGF-β/RII) in contrast to un-supplemented or individually supplemented groups, respectively. According to this study, TQ increased the chemopreventive property of Vit.D during the beginning phase of colon cancer in rat with the possible to repress development of pre-neoplastic injuries in colon carcinogenesis (32).

Table 1 provides a brief summary of the anti-cancer activity of TQ against alimentary canal cancers.

Liver cancer

Liver cancer accounts 5th the most common malignancy universal and the 3th leading cause of death. Hepatocellular carcinoma (HCC) is responsible for about 80-90% of all liver cancer and accounts as the 4th most common reason of

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Table 1. Effect of TQ on alimentary canal cancer

Animal/Cell	Mechanism	Ref.
Oral cancer cells (T28)	Increased apoptosis (down regulation of p38β MAPK)	(13)
Eesophageal carcinoma cell	Enhanced chemosensitivity and cellular apoptosis of DDP (blocking the activation of JAK2/STAT3 way)	(14)
Ggastric cancer cells, Xenograft tumor mouse model	Elevated the apoptotic features in gastric cancer cells induced by 5-FU (decreasing of bcl-2, increasing of bax as well as caspase-3 and caspase-9 activation)	(18)
Human gastric cancer cells (HGC27), xenograft tumor in mice model	Caused to apoptotic cell death, prevented the STAT3 phosphorylation, decreased the STAT3-regulated genes expression including cyclin D, survivin, Bcl-2, and VEGF, and activated caspase-3,7,9.	(19)
Rat colorectal cancer	5-FU/TQ compound caused to an more important decrease colorectal tumors and large improper crypts foci than individual drugs therapy, 5-FU/TQ significantly collaborated to suppress the expression of procancerous Wnt, NF-κB, β-catenin, iNOS, COX-2, VEGF and TBRAS and increase anti-tumorigenesis TGF-β1, TGF-βRII, DKK -1, CDNK-1A, GPx and Smad4 expression.	(23)
SW-626 human colon cancer cell line	Cellular damages and intervention of cellular metabolic actions	(24)
C26 mouse colorectal carcinoma spheroids	Decreased the sizes and numbers of ACF and tumor multiplicity, reduced C26 cell invasion and repressed three-dimensional spheroids Growth	(25)
Human colon cancer HCT116 cells	Caused to apoptosis (inactivation of STAT3 through stopping JAK2-and Src□mediated phosphorylation of EGF receptor tyrosine kinase)	(26)
Colon cancer cells	Reduced cell viability in HCT116 and COLO205 cells, reduced phosphorylated p65 level in the nucleus, prevention of NF-Kb action, reduced c-Myc, Bcl-2 and VEGF levels expression, powered the induction of cell death	(27)
Human colon cancer cell lines	Enhanced the effectiveness of topotecan as a the chemotherapeutic reagent (preventing of proliferation and decreasing toxicity through p53- and Bax/Bcl2-independent mechanisms)	(28)
Human LoVo colon cancer cell	Decreased human LoVo colon cancer cell proliferation and migration (decreasing prostaglandin E2 induced COX-2 activation)	(29)
Colon carcinogenesis in rats	Ameliorated entirely oxidative stress induced by DMH at beginning and stable histo-logical alternations and tumor promotion, abolished oxidative stress aggravation at begining, decreased tumour occurrence	(30)
Mice	Interfered with polyp development in mice (induction of specific apoptosis of tumor-cell and through modulating Wnt signaling)	(31)
Colon cancer in rat	Increased the chemopreventive property of Vit.D during the beginning phase of colon cancer, decreased of the grown tumors numbers and major improper crypts foci, reduction of pro-cancerous molecules (β-catenin, NF-κB, iNOS, Wnt, VEGF, COX-2 and HSP-90), a great increase of anti-tumorigenesis biomarkers (DKK-1, TGF-β1, smad4, CDNK-1A and TGF-β/RII)	(32)

cancer mortality. However there are various planes for liver cancer treatment, chemoprevention appears to be the best plan for decrease the occurrence of this disease (33, 34).

In HCC, interleukin-8 (IL-8) is increased, therefore it is considered as a target for treatment. However the transcription factor NF-κB adjusts expression of IL-8, and while TQ prevents NF-κB activity. The careful mechanisms that with them TQ regulates IL-8 and cancer cell proliferation remain to be explained. Ashour et al. reported that TQ prevented HCC cells proliferation in a dose and time dependent way, resulted to G2M cell cycle stop, and motivated apoptosis. Treatment with TQ inhibited NF-κB expression and repressed IL-8 and its receptors. Also, treatment with TQ resulted to enhanced levels of ROS and genes involved in oxidative stress. Pretreatment of HepG2 cells with N-acetylcysteine as a remover of ROS, inhibited TQ-induced cell death. TQ increased mRNA expression of pro-apoptotic Bcl-xS, and prevented expression of the anti-apoptotic gene Bcl-2. TQ increased TRAIL-induced death of HepG2 cells, partly through increasing in TRAIL death receptors, preventing IL-8 and NF-κB and inducing apoptosis. Generally, this study provided views into the molecular mechanisms of TQ-dependent repression of HCC cell proliferation and emphasized its ability as anti-HCC drug (35).

The other study, investigated antitumor activity of TQ through examination of its effect on cell cycle, angiogenesis, and apoptosis. The findings showed the ability of TQ as an antitumor agent against various types of cancer such as colon, brain, cervix, and liver at both a time- and concentration-dependent way. It induced G2/M phase cell cycle stop in HepG2 cells, and a concentration-dependent development in the number of apoptotic cells with an elevation in the ratio of Bax/BCL-2. Furthermore, mRNA expression and protein level of VEGF reduced when the concentration of TQ elevated. The data of this study indicated an increase in the glutathione content and phase II enzyme GST activity in HepG2 cells. This study, supported the beneficial effect of TQ as a chemopreventive and therapeutic agent against liver cancer (36).

Sayed-Ahmed et al. investigated whether TQ could inhibit beginning of hepatocarcinogenesis which was induced by diethylnitrosamine (DENA) as a strong initiator and hepatocarcinogen in rat model. Administration of TQ (4 mg/kg/day, orally) completely corrected biochemical and histopathological alternations following DENA injection to the control values. The findings of this study suggested reduced mRNA expression of glutathione peroxidase (GSHPx), catalase (CAT) and glutathione-s-transferase (GST) during DENA-

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induced beginning of hepatic carcinogenesis that TQ supplementation inhibited the progression of DENA-induced initiation of liver cancer through reducing oxidative stress and maintaining the mRNA expression of GSHPx and CAT and activity of antioxidant enzymes (37).

Agents that prevent cell multiplication and limit hepatic tumorigenesis by cell cycle adjustment have a positive property in the HCC treatment. Raghunandhakumar and colleaguees investigated the effect of TQ on N-nitrosodiethylamine (NDEA) - induced hepatocarcinogenesis in rat. TQ (20 mg/kg) treatment markedly decreased liver damage markers and reduced tumor markers and inhibited hepatic nodule formation and decreased tumor proliferation in liver cancer induced by NDEA which was obvious from argyrophilic nucleolar organizer region (AgNORs) dying. In addition, TQ treatment importantly decreased adverse changes through stopping of cell proliferation that greatly caused to G1/S stop in the cell cycle transmission. According to this study, TQ has a potential anti proliferative effect through adjusting the G1/S phase cell cycle transmission and showed a beneficial effect in the HCC treatment (38).

Table 2 provides a brief summary of the anti-cancer activity of TQ against hepatic cancers.

Pancreatic cancer

Pancreatic cancer which is known as one of the most fatal cancers in the world, keeps on to be resistant to any treatment approaches.

Banerjee et al. for the first time, investigated chemosensitizing effect of TQ to ordinary chemotherapeutic agents by application of an orthotopic model of pancreatic cancer both *in vitro* and *in vivo*. *In vitro* findings showed that preexposure of cells with TQ (25 mumol/L) for 48 hours after oxaliplatin or gemcitabine caused to in 60% to 80% development prevention in contrast to 15% to 25% when oxaliplatin or gemcitabine was applied alone. Also, TQ could power the pancreatic cancer cells death which was induced by chemotherapeutic agents through down-regulation of NF-kB, NF-kB-dependent antiapoptotic genes and Bcl-2 family. In addition, markedly, TQ was able to decrease in NF-kB (which gets activated on exposure of pancreatic cancer cells to ordinary chemotherapeutic agents) *in vitro*, causing in chemosensitization. In addition, *in vitro* findings indicated that TQ with oxaliplatin and/or gemcitabine is much further helpful as an antitumor agent in contrast to either agent alone. According to this study, particular target, such as NF-kB, was inactivated in animal tumors which was pretreated by TQ after oxaliplatin and/or gemcitabine. These results explained that TQ can cancel oxaliplatin and/or gemcitabine induced activation of NF-kB, causing to in the

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chemosensitization of pancreatic tumors to usual therapeutics (39).

In the other study, Yusufi and colleagues indicated high anti-proliferative effect, superior chemo-sensitizing activity of TQ analogs against pancreatic cancer *in vitro* and in mixed with Gemcitabine as an effective drug in pancreas cancer treatment (40).

In pancreatic cancer, the expression of high molecular weight glycoprotein mucin 4 (MUC4) is increased which has role in the regulation of growth, differentiation, metastasis, and the chemoresistance of PC cells. Prevention of MUC4 expression in the normal pancreatic ductal cells is a promising target for new cancer therapies. In this context, Torres et al. investigated TQ effect on PC cells and particularly evaluated its effect on MUC4 expression. The results showed that TQ treatment decreased expression of MUC4 through the proteasomal way and caused to apoptosis in PC cells. Reduce in MUC4 expression associated with an increase in apoptosis, reduced motility, and reduced emigration of PC cells. According to this study, TQ has potential effect for the development of new treatment against pancreatic cancer (41).

Other study surveyed the anti-metastatic property of TQ on the pancreatic cancer both *in vitro and in vivo*. The findings indicated that TQ repressed the emigration and invasion of Panc-1 cells does-dependently. TQ greatly decreased matrix metallopeptidase 9 (MMP-9) and NF-kB in Panc-1 cells. Also, TQ administration significantly decreased tumor metastasis in contrast to untreated control in metastatic model induced in mice. In addition to, MMP-9 and NF-kB expression was suppressed following TQ treatment. The findings of this study indicated that TQ has anti-metastatic activity on pancreatic cancer both *in vitro* and *in vivo*, that possibly related to decrease in NF-kB expression and its regulated molecules such as MMP-9 protein. Based on these results, TQ could be introduced as an antimetastatic agent human pancreatic cancer treatment (42).

Inflammation has been introduced as an important factor in increase of the solid tumour malignancies. Pancreatic ductal adenocarcinoma (PDAC) is almost resistant to the treatments. Chronic pancreatitis including both congenital and sporadic are related to an enhanced risk of increasing PDA. Chehl et al. indicated that TQ caused to apoptosis and prevented PDA cells proliferation. In addition, the findings of this study showed antiinflammatory properties of TQ in PDA cells. According to these findings, TQ as a new preventer of proinflammatory pathways has a good strategy that

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mixes anti-inflammatory and proapoptotic modes of activity (43).

Other study surveyed antineoplastic effect of TQ in human PDAC cell lines, MiaPaCa-2 and AsPC-1. TQ (10-50 μM) prevented cell viability and multiplication and resulted partial G2 cycle stop in dependently dose manner in both cell lines. Accumulation of the cells in subG0/G1 phase, showing apoptosis. This was related to increase of p53 and decrease of Bcl-2. *In vivo*, TQ greatly decreased tumor size in 67% of defined tumor xenograft. This study exhibited that TQ mediated posttranslational regulation of histone acetylation, prevented expression of HDACs, and caused to proapoptotic signaling ways. The mentioned molecular targets show reason for application of TQ as a promising antineoplastic agent to inhibit postoperative cancer return and to delay viability of PDAC patients after operation resection (44).

The study was done by Wang showed the anti-neoplastic and anti-metastatic properties of TQ on the human pancreatic carcinoma in nude mice and it explained that these effects may be related to the decrease in expressions of XIAP and MMP-9 (45).

Table 2. Effect of TQ on liver cancer

Animal/Cell	Mechanism	Ref.
Human hepatocellular carcinoma cell	Prevented HCC cells proliferation, resulted to G2M cell cycle stop, and motivated apoptosis, inhibited NF-κB expression and repressed IL-8 and its receptors, enhanced levels of ROS and genes involved in oxidative stress, increased mRNA expression of pro-apoptotic Bcl-xS, and prevented expression of the anti-apoptotic gene Bcl-2	(35)
HepG2 cells	Induced G2/M phase cell cycle stop, developed the number of apoptotic cells with an elevation in the ratio of Bax/BCL-2, reduced mRNA expression and protein level of VEGF	(36)
Rat	Inhibited the progression of DENA-induced initiation of liver cancer (reducing oxidative stress and maintaining mRNA expression of GSHPx and CAT and activity of antioxidant enzymes)	(37)
Rat	Decreased liver damage markers and reduced tumor markers and inhibited hepatic nodule formation and decreased tumor proliferation in liver cancer induced by NDEA, decreased adverse changes (stopping of cell proliferation that greatly caused to G1/S stop)	(38)

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Table 3. Effect of TQ on pancreatic cancer

Animal/Cell	Mechanism	Ref.
PC cell, pancreatic cancer in mice	Powered the pancreatic cancer cells death which was induced by chemotherapeutic agents (down-regulation of NF-κB , NF-κB -dependent antiapoptotic genes and Bcl-2 family)	(39)
PC cells	Anti-proliferative effect, superior chemo-sensitizing activity	(40)
PC cells	Decreased expression of MUC4 through the proteasomal way and caused to apoptosis, reduced motility, and reduced emigration of PC cells	(41)
Panc-1 cells, Metastatic model induced in mice	Repressed the emigration and invasion of Panc-1 cells, decreased MMP-9 and NF-κB in Panc-1 cells. Also, TQ administration significantly decreased tumor metastasis, suppressed MMP-9 and NF-κB expression	(42)
PDA cells	Caused to apoptosis and prevented PDA cells proliferation, it showed antiinflammatory properties in PDA cells	(43)
Human PDA, *In vivo*	Prevented cell viability and multiplication and resulted partial G2 cycle stop, acumulation of the cells in subG0/G1 phase, showing apoptosis (increase of p53 and decrease of Bcl-2), decreased tumor size, inhibition of histone deacetylation, caused to proapoptotic signaling ways	(44)
Human pancreatic carcinoma in nude mice	Showed anti-neoplastic and anti-metastatic properties (decrease in expressions of XIAP and MMP-9)	(45)

Conclusion

Medicinal plants have been used as a curative treatment agent for their many benefits such as ability to treat diseases including cancers. TQ (active constituent of *N. sativa*) has been considered in traditional medicine for treating different cancers related to GI system. This review indicated the *in vitro* and *in vivo* anti- cancer activity of TQ associated with its possible mechanisms in GI cancers including oral cancer, esoghageal cancer, gastric cancer, colorectal cancer and also liver and pancreatic cancers. The different mechanisms of anticancer effects of TQ including cell cycle arrest, suppression of cell proliferation and differentiation, increasing apoptosis in the cells by increasing of BAX and p53 expression and downregulation of Bcl-2 and Bcl-xl and decreased cells viability. According to this review TQ and/or its analogues can act as an anticancer agent alone or in combination with chemotherapeutic drugs in treatment of GI tract cancers, however well designed clinical trials in humans are required to confirm these effects.