Original Article

# The Assessment Of The Complementary Chemotherapeutic Potential Of Nigella Sativa In Patients With Colorectal Cancer: A Randomized Clinical Trial

**Davoud Oladdameshghi[1], talieh mollabashi [2], Alireza Akhzari[3], Abolfazl Mohammadbeigi[4], Masoumeh Akbari[5], Mohamad hasan haj Rahimian Tasuji[6], Samira Khani[7], Hossein Moradi[8], Ahmad Abolhasani[9], Hoda Abolhasani*[10]**

1: Hematologist & Medical Oncologist, Clinical research development, Qom University of Medical Sciences, Qom, Iran
2: Medical internist student, Clinical research development, Qom University of Medical Sciences, Qom, Iran
3: Master student of genetic, Faculty of Science, Department of biology, East Tehran Branch, Islamic Azad University, Tehran, Iran
4: Research center for Environmental pollutants, Professor of Epidemiology, Department of Epidemiology and Biostatistics, Faculty of health, Qom University of Medical Sciences, Qom, Iran
5: Spiritual Health Research Center, Faculty of Paramedical, Qom University of Medical Sciences, Qom, Iran
6: Spiritual Health Research Center, Qom University of Medical Sciences, Qom
Researcher and expert research Quran and etrat student vice chancellor, qom, university of medical sienicer, qom, iran
7: Neurosciences Research Center, Qom university of medical sciences, Qom, Iran
8: School of Traditional Medicine,Qom university of Medical Science,Qom, Iran.
9: Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran
Spiritual Health Research Center, Qom University of Medical Sciences, Qom, Iran;
Department of Pharmacology, School of Medicine, Qom University of Medical Sciences, Qom
10: Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran;
Spiritual Health Research Center, Qom University of Medical Sciences, Qom, Iran;
Department of Pharmacology, School of Medicine, Qom University of Medical Sciences, Qom, Iran

Corresponding author
* Hoda Abolhasani; Alghadir Boulevard, opposite of Yadegar Emam Stadium, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran , E-mail: hodaabolhasani@gmail.com
**DOI:** 10.47750/pnr.2023.14.04.65

## Abstract

**Background:** Colorectal cancer (CRC) is among the leading cause of cancer-related morbidity worldwide. Surgery and chemotherapy are the first-line therapy for CRC patients; however, the efficacy and side effects are two major concerns. Nigella sativa (NS) has been found to exhibit anti-cancer effects in numerous in vitro studies. The present study aimed to evaluate the potential therapeutic effect of NS on CRC patients.

**Methods:** In this randomized trial, a total of 39 patients with Stage IIb and III CRC who underwent colon surgery were randomly assigned to receive routine chemotherapy drugs in combination with 900 mg/day of NS (Group 1, n=18) or only chemotherapy drugs (Group 2, n=21). CRC blood biomarkers including CEA and CA19-9 were measured in both groups before, in the middle, and at the end of treatment in addition to obtaining demographic characteristics.

**Results:** We observed that NS combined with chemotherapy reduced the CA19-9 serum level in the intervention group when compared with the baseline level before treatment. However, there was no significant difference between CA19-9 serum levels in the intervention group compared with the control group at all time points. Moreover, CEA did not show any reduction in the intervention group compared with the control group.

**Conclusion:** Except for the NS effect on CA19-9 level in intervention group after therapy compared to baseline, NS is not effective on CRC biomarkers with this concentration and follow-up period. Therefore, longer treatment durations and monitoring with higher doses are probably required.

**Keywords:** Nigella sativa, Colorectal cancer(CRC), Complementary therapy, CEA, CA19-9

## 1. INTRODUCTION:

Colorectal cancer (CRC), the second leading cause of cancer mortality and 10% of annual cancer new cases, impose a major health burden on the patients [1, 2]. Surgery and chemotherapy have long been used as a realistic strategy in the fight against cancers.[3] However, many CRC patients develop recurrence or suffer from side effects of current therapies. [4,5] Therefore, by considering the treatment burden in CRC, developing effective and safe treatments is a pivotal unmet clinical need.

From ancient times, herbal medicines have always been considered as a promising pharmaceutical compound in the treatment and prevention of various diseases. [6-8] Numerous widely-used antitumor agents have been obtained from natural sources. [9,10] Emerging evidence has shown that natural products may serve as potential cancer therapy enhancers via the impact on multiple targets and signaling pathways.[11] Many studies suggest that a diet with many beneficial substances from fruits, vegetables, cereal grains, and spices is helpful to protect against many cancers. [12,13]

Nigella sativa (NS) is a miraculous plant, commonly known as black seed or Kalonji, and belongs to the family Ranunculaceae herbaceous. [14] NS is considered a promising medicinal plant displaying health benefits both in vitro and in vivo.[15,16] Thymoquinone (TQ), the main bioactive ingredient of NS oil, plays an indispensable role against inflammatory diseases and cancer by epigenetic regulation, modulation of key signaling effectors involved in inflammation and cancer progression, hindering cell proliferation and survival, invasion and metastasis.[17] Moreover, TQ exerts synergistic effects with anticancer agents while diminishing their adverse effects by elevating the level of anti-oxidant enzyme. [18] The anticancer properties of TQ has been documented in various cancer cell lines including those from CRC [19-21] prostate, skin, breast, kidney, gastric cancer, cholangiocarcinoma, Hepatocellular carcinoma, renal cell carcinoma, T-cell leukemia and ovarian cancer. [22] It is indicated that TQ induce cell cycle arrest in Jurcat cells [23], lung cancer[24] and prostate cancer. [25] TQ has shown the ability to significantly inhibit the growth of MV4-11 AML cells through hypomethylation and therefore re-expression of JAK/STAT negative regulators. [26] Moreover, it is demonstrated that TQ inhibits cell proliferation, invasion and tumor growth by inhibition of eukaryotic elongation factor 2 kinase (eEF-2K) in the most aggressive and chemoresistant subtype of breast cancer, Triple-negative breast cancer. [27] On the other hand, TQ induce cell cycle arrest in CRC cell lines. [28,29] It is shown that TQ chemosensetize CRC cells to imatinib as well via modulation of the genes involved in drug uptake.[30] Moreover, TQ showed to hamper tumor growth and progression in murine models of colon cancer. [17,31] On the other hand, multiple studies have well documented that Carcinoembryonic antigen (CEA) and Carbohydrate antigen 19-9 (CA19-9) are the most critical CRC biomarkers which closely associated with therapy outcome and patient prognosis. [32,33] Therefore, we aimed to carry out a randomized clinical trial on the effect of NS on CRC patients and explore the potential chemotherapeutic effect of NS using examining serum CA19-9 and CEA levels.

## 2. MATERIALS AND METHODS:

### 2.1 Study setting

This open-labeled randomized clinical trial was carried out in Shahid Beheshti Hospital, Qom, Iran. A total of 39 CRC patients diagnosed with stages IIb and III from August 2019 to February 2020 with colon surgery history were included (Figure 1).

After identification of the patients, participants were randomized using computer based assignment model, to receive either therapy with NS combined with chemotherapeutic agent (intervention group) or only chemotherapeutic agent (control group). Independent of intervention type and outcome evaluation, sequence generation and allocation concealment was performed. The sample size was calculated according to HebatAlla Fathi et al. study.[34]

More specifically, the patients were assigned to two groups. Group 1 (intervention group) included 18 CRC patients treated with a first-line CRC treatment regimen including FOLFOX regimen (oxaliplatin, 5-fluorouracil, and folinic acid), rarely FOLFIRI regimen (irinotecan, 5-fluorouracil, and folinic acid) both in combination with NS capsule, containing 900 mg of black seed powder, three times a day. Group 2 (control group) included 24 patients who were allocated to receive FOLFOX regimen and rarely FOLFIRI regimen. The duration of treatment was 180 to 240 days. In both groups, blood biomarkers including CEA and CA19-9 were measured before, middle (three months), and at the end (six months) of treatment.

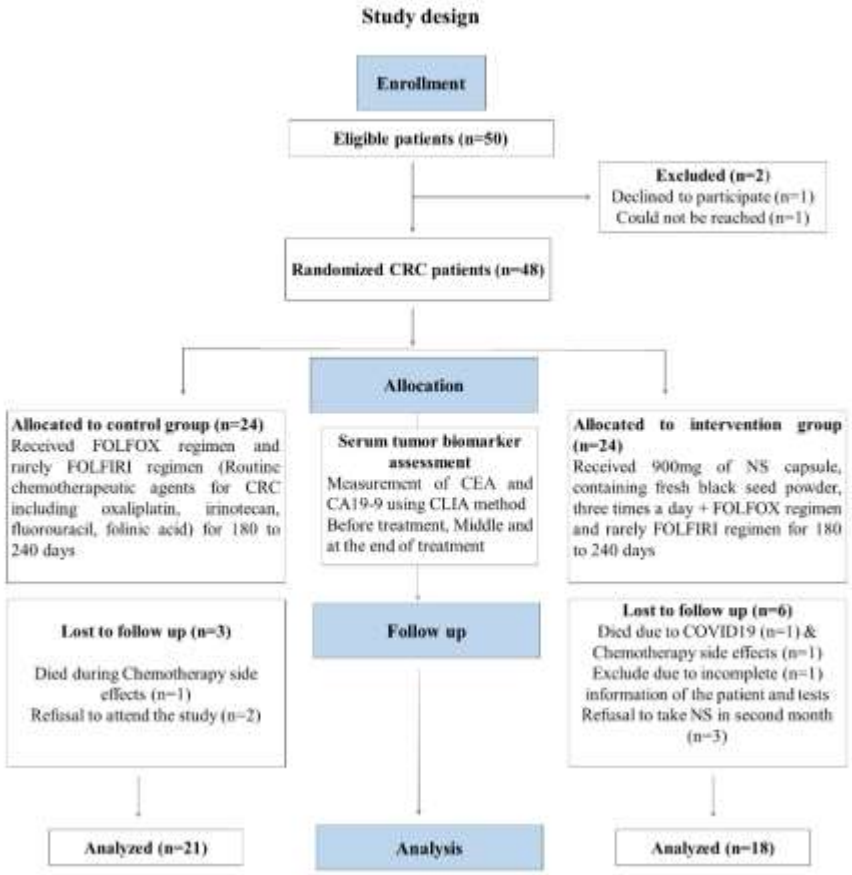


Figure 1. Study design.

## 2.2 Inclusion criteria and exclusion criteria

The inclusion criteria were as follows: (1) The age between 20 to 70 years old; (2) Patients diagnosed with Stage IIb/III; (3) Colon surgery after diagnosis of CRC; (4) Patients before first line chemotherapy. (5) informed consent; (6) absence of exclusion criteria.

The exclusion criteria were (1) patients with intolerance to black seed; (2) allergies; (3) severe hypotension; (4) menorrhagia; (4) neurological and physical complications; (5) pregnant and lactating women; (6) married women of childbearing age; (7) those without reliable contraceptive methods.

## 2.3 Ethical considerations

Written informed consent was obtained on enrollment and the study was approved by the medical ethics board of Qom University of Medical Science, Qom, Iran with a dedicated code of ethics committee: IR.MUQ.REC.1397. 15. After approval of the proposal, the clinical trial code (IRCT20180513039641N1) has been registered. All methods were conducted according to the instructions and regulations.

## 2.4 Capsule preparation

The black seeds after soaking in vinegar overnight in order to maintain TQ in black seeds. Then, the black seeds were dried and then powdered. Next, 900mg of them were placed in capsules. The capsules were freshly prepared each month.

## 2.5 General characteristics

General characteristics such as sex, age, body mass index (BMI), and glomerular filtration rate (GFR) were collected.

## 2.6 Serum tumor biomarker assessment

In order to assess the potential effect of NS, the serum level of tumor biomarkers including CEA and CA19-9 were measured for each patient at three time points: baseline, middle of the treatment (3 months) and at the of the treatment (6 months) using DiaSource kits (DIAsource ImmunoAssays®, Belgium) by an automatic chemiluminescence immunoassay (CLIA).

## 2.7 Statistical analysis

Statistical analysis was performed by GraphPad Prism 9.0.0. In order to compare each time, point between control and intervention group, the data with Gaussian distribution were analyzed using unpaired t-test or its equivalent, Mann–Whitney U test, when Shapiro-Wilk test did not show normal distribution. Moreover, one-way ANOVA or its equivalent, Kruskal-Wallis was used to compare various time points. Two-way ANOVA was performed to determine the interaction effect. The data were expressed as mean ± standard deviation. The p-value ($P < 0.05$) was considered significant. Frequency and percentage were used for qualitative variables.

# 3. RESULTS:

## 3.1 Patient Characteristics

In this randomized clinical trial study, 39 CRC patients were enrolled. The gender distribution of the patients is presented in Table 1. 18 patients out of the 39 patients (46.2%) who were enrolled in our study were allocated to case group (including 11 males and 7 females). Other 21 patients (53.8%) were allocated to the control group (including 13 men and 8 women) who only received chemotherapy drugs.

**Table 1.** Gender distribution of study population

| Sex | No. | Percent | Valid percentage |
|---|---|---|---|
| Male | 24 | 61.5 | 61.5 |

| | | | |
|---|---|---|---|
| Female | 15 | 38.5 | 38.5 |
| Total | 39 | 100 | 100 |

## 3.2 Age, BMI and GFR distribution:

There were no significant differences when study groups were compared according to their average age, BMI and GFR (Table 2).

**Table 2**. BMI and GFR distribution in study population

| Variable | group | No. | Mean ± SD | P-value |
|---|---|---|---|---|
| Age | Intervention | 18 | 59.24 ±10.11 | 0.38 |
| | Control | 21 | 56.05±11.9 | |
| BMI | Intervention | 18 | 24.46±3.49 | 0.26 |
| | Control | 21 | 25.45±1.89 | |
| GFR | Intervention | 18 | 80.57±14.04 | 0.34 |
| | Control | 21 | 85.18±15.35 | |

## 3.3 Serum level of tumor biomarkers

The serum level of CRC biomarkers including CEA and CA19-9 were measured at three time points by CLIA method. The figures for CEA before treatment and in the middle of treatment were found to be significantly higher in the control group compared to the intervention but still remained in standard normal range (Table 3). Nevertheless, the serum level of CEA after treatment remained unchanged when compared between the control and the intervention groups (p.value>0.05) (Table 3).

Moreover, there was no significant difference in CEA level when a comparison between three time-point was performed in each group (Figure 2). On the other hand, there was no significant difference between the CA19-9 serum levels of the control and the intervention groups at all three time points (Table 3). However, a declining trend was found in the serum levels of CA19-9 in intervention group compared with control group (Figure 3). No significant interaction effect was observed when Two-way ANOVA was performed for CEA and CA19-9.

**Table 3.** The effect of NS on colorectal cancer biomarkers biomarkers

| **Colorectal cancer biomarkers** | **Intervention group (n=18)** | **Control group (n=21)** | **P-value** |
|---|---|---|---|
| **CEA** | | | |
| Before treatment | 3.5±1.56 | 2.93±2.81 | 0.012 |
| Middle of treatment | 4.28 ± 1.72 | 2.47 ± 1.06 | 0/0004 |

| | | | |
|---|---|---|---|
| End of treatment | 3.68±1.03 | 3.6 ±1.86 | 0/82 |
| **CA19-9** | | | |
| Before treatment | 8.71±6.2 | 6.39±5.3 | 0.08 |
| Middle of treatment | 6.15± 4.81 | 9.87±9.57 | 0.67 |
| End of treatment | 4.8±3.26 | 6.54±6.05 | 0.9 |

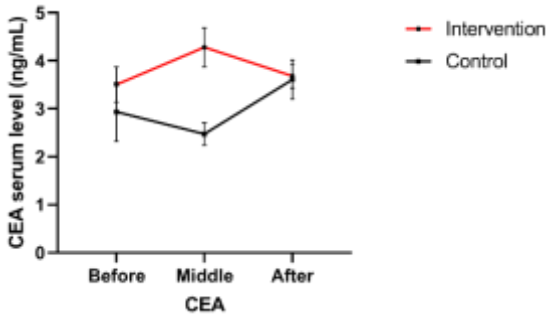


**Figure 2. The CEA serum level at different time points.** In order to compare the serum levels of the CEA before, middle and after the treatment in each study group, the Kruskal-Wallis test was conducted. No significant difference was observed between these three timepoints.

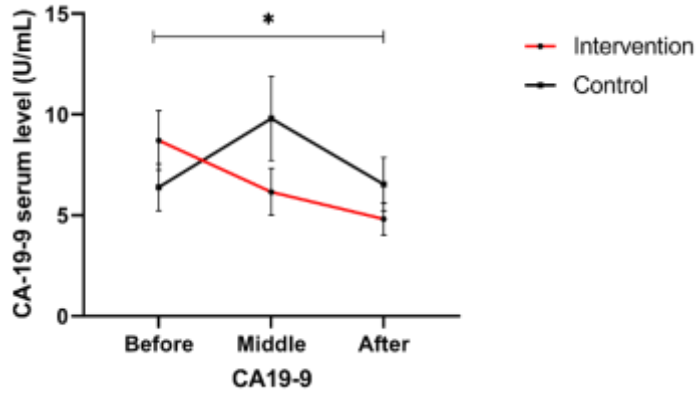


**Figure 3. The CA19-9 serum level at different time points.** In order to compare the serum levels of the CA19-9 before, middle and after the treatment in each study group, the Kruskal-Wallis test was carried out. It is shown that the CA19-9 levels was significantly decrease after treatment in the intervention group when compared to the control group (p.value: 0.015)

## 4. DISCUSSION:

Natural products have historically been considered a vital source for curing numerous kinds of diseases and remained a widely applied form of supplementary and alternative therapies in combination with chemotherapy or radiotherapy to improve the efficacy of cancer treatment.[35-37] According to cell culture studies and several animal models, NS has proved to possess strong antioxidant, anti-inflammatory, analgesic, antipyretic, antibacterial, antifungal, anti-cancer, and immunopotentiating activities and plays role in preventing and treating numerous diseases without any adverse effects. [38-40] and NS is rich in bioactive constituents like thymoquinone, sterols and saponins, alkaloids, phenolic compounds, and saponin. These bioactive constituents not only play indispensable roles in tumor prevention but also in eliminating the side effects of chemotherapy agents. [41] Another study revealed that NS resulted in tumor destruction by induction of apoptosis in tumor cells, but had no effects on various normal cells.[42] Hence, complementary chemotherapy can be employed as new therapeutic opportunities for CRC treatment. Mousa et al. in a randomized pretest-post-test control group specified that consuming 5 grams of black seed per day reduced Febrile neutropenia and Length of hospital stay in children with brain tumors improving their quality of life. [34]

The current study was the first clinical trial to investigate NS cancer activities in CRC patients. CEA and CA19-9 had sensitivity and specificity in investigating the CRC treatment process and recommended that simultaneous evaluation of CEA and CA19-9 may exhibit more accurate results about the disease process.[43] Therefore, we sought to evaluate the outcome using measurement of CEA and CA19-9 at three different time points including before, in the middle, and at the end of chemotherapy. Our finding showed that NS exerts a significant effect on the reduction of a specific CRC tumor marker, CA19-9. In fact, statistical analysis showed that the CA19-9 serum level was significantly reduced after therapy, compared with the baseline level in intervention group ($p<0.05$).

However, there was no significant difference between the CA19-9 serum levels of the control and the intervention groups at all three time points. It might be due to the low receiving dose of NS. On the other hand, the CEA levels before and at the middle of treatment was found to be significantly higher compared to the intervention group, but still remained in the standard normal range. We hypothesized that it might be due to metastasis. Our data are in line with preliminary investigations on NS exhibiting an inhibitory effect on cancer cells. H. Sarman et al. evaluated the interactions between TQ, a bioactive compound in NS, with fluorouracil and oxaliplatin in osteosarcoma cells. They found that TQ has potential benefits in preventing the onset and progression of chemotherapy drug-induced toxicity and may reduce resistance to chemotherapy agents.[44]

In another study, Korak et al. reported that NS has anticancer effects due to its remarkable immunomodulatory effects.[45] Very few clinical studies have investigated the interactions between NS in combination with other drugs in clinical research.

This study was a pioneer investigation aiming at the evaluation of the effect of NS on CRC biomarkers in the human. However, our study had some limitations. The serum level of CEA before treatment initiation was significantly higher in the intervention group compared to the control group however no significant difference was found at the end of treatment. Moreover, the small sample size was another limitation.

Concerning the interaction effect of CEA and CA19-9 in three time-points, NS is not effective on CRC biomarkers with this concentration and follow-up period. This was the first study on NS that may open an avenue in the future for plant-based pharmaceuticals. However, further studies are needed to evaluate the NS effective therapeutic doses and the underlying mechanism in cancer patients with larger sample size. It is suggested that various higher concentrations of NS in a larger sample size should be investigated with a more extended follow-up period of patients after treatment.

## ACKNOWLEDGEMENTS

We would like to appreciate all the patients for their cooperation in this investigation.

## REFERENCES:

1. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Translational Oncology. 2021;14(10):101174.
2. Simon K. Colorectal cancer development and advances in screening. Clinical interventions in aging. 2016;11:967.
3. Xie Y-H, Chen Y-X, Fang J-Y. Comprehensive review of targeted therapy for colorectal cancer. Signal transduction and targeted therapy. 2020;5(1):1-30.
4. Ryuk JP, Choi G-S, Park JS, Kim HJ, Park SY, Yoon GS, et al. Predictive factors and the prognosis of recurrence of colorectal cancer within 2 years after curative resection. Annals of surgical treatment and research. 2014;86(3):143-51.
5. Lawler M, Alsina D, Adams RA, Anderson AS, Brown G, Fearnhead NS, et al. Critical research gaps and recommendations to inform research prioritisation for more effective prevention and improved outcomes in colorectal cancer. Gut. 2018;67(1):179-93.
6. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery. 2021;20(3):200-16.
7. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559.
8. Mahmoudi S, Ghorbani M, Sabzichi M, Ramezani F, Hamishehkar H, Samadi N. Targeted hyaluronic acid-based lipid nanoparticle for apigenin delivery to induce Nrf2-dependent apoptosis in lung cancer cells. Journal of Drug Delivery Science and Technology. 2019;49:268-76.
9. Cragg GM, Pezzuto JM. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. Medical Principles and Practice. 2016;25(Suppl. 2):41-59.
10. Iqbal J, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA, et al. Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine. 2017;7(12):1129-50.
11. Agbarya A, Ruimi N, Epelbaum R, Ben-Arye E, Mahajna J. Natural products as potential cancer therapy enhancers: A preclinical update. SAGE open medicine. 2014;2:2050312114546924.
12. Dhingra D, Michael M, Rajput H, Patil R. Dietary fibre in foods: a review. Journal of food science and technology. 2012;49(3):255-66.
13. Sardana RK, Chhikara N, Tanwar B, Panghal A. Dietary impact on esophageal cancer in humans: a review. Food & function. 2018;9(4):1967-77.
14. Rahmani AH, Alzohairy MA, Khan MA, Aly SM. Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways. Evidence-Based Complementary and Alternative Medicine. 2014;2014.
15. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific journal of tropical biomedicine. 2013;3(5):337-52.
16. Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. Nigella sativa L.(black cumin): a promising natural remedy for wide range of illnesses. Evidence-Based Complementary and Alternative Medicine. 2019;2019.
17. Gali-Muhtasib H, Ocker M, Kuester D, Krueger S, El-Hajj Z, Diestel A, et al. Thymoquinone reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models. Journal of cellular and molecular medicine. 2008;12(1):330-42.
18. Schneider-Stock R, Fakhoury IH, Zaki AM, El-Baba CO, Gali-Muhtasib HU. Thymoquinone: fifty years of success in the battle against cancer models. Drug discovery today. 2014;19(1):18-30.
19. Hsu H-H, Chen M-C, Day CH, Lin Y-M, Li S-Y, Tu C-C, et al. Thymoquinone suppresses migration of LoVo human colon cancer cells by reducing prostaglandin E2 induced COX-2 activation. World journal of gastroenterology. 2017;23(7):1171.
20. Fröhlich T, Ndreshkjana B, Muenzner JK, Reiter C, Hofmeister E, Mederer S, et al. Synthesis of novel hybrids of thymoquinone and artemisinin with high activity and selectivity against colon cancer. ChemMedChem. 2017;12(3):226-34.
21. Zhang L, Bai Y, Yang Y. Thymoquinone chemosensitizes colon cancer cells through inhibition of NF-κB. Oncology letters. 2016;12(4):2840-5.
22. Gomathinayagam R, Ha JH, Jayaraman M, Song YS, Isidoro C, Dhanasekaran DN. Chemopreventive and anticancer effects of thymoquinone: cellular and molecular targets. Journal of cancer prevention. 2020;25(3):136.
23. Alhosin M, Abusnina A, Achour M, Sharif T, Muller C, Peluso J, et al. Induction of apoptosis by thymoquinone in lymphoblastic leukemia Jurkat cells is mediated by a p73-dependent pathway which targets the epigenetic integrator UHRF1. Biochemical pharmacology. 2010;79(9):1251-60.
24. Jafri SH, Glass J, Shi R, Zhang S, Prince M, Kleiner-Hancock H. Thymoquinone and cisplatin as a therapeutic combination in lung cancer: In vitro and in vivo. Journal of Experimental & Clinical Cancer Research. 2010;29(1):1-11.
25. Kaseb AO, Chinnakannu K, Chen D, Sivanandam A, Tejwani S, Menon M, et al. Androgen receptor–and E2F-1–targeted thymoquinone therapy for hormone-refractory prostate cancer. Cancer research. 2007;67(16):7782-8.
26. Al-Rawashde FA, Johan MF, Taib WRW, Ismail I, Johari SATT, Almajali B, et al. Thymoquinone inhibits growth of acute myeloid leukemia cells through reversal SHP-1 and SOCS-3 hypermethylation: In vitro and in silico evaluation. Pharmaceuticals. 2021;14(12):1287.
27. Kabil N, Bayraktar R, Kahraman N, Mokhlis HA, Calin GA, Lopez-Berestein G, et al. Thymoquinone inhibits cell proliferation, migration, and invasion by regulating the elongation factor 2 kinase (eEF-2K) signaling axis in triple-negative breast cancer. Breast Cancer Research and Treatment. 2018;171(3):593-605.
28. Wirries A, Breyer S, Quint K, Schobert R, Ocker M. Thymoquinone hydrazone derivatives cause cell cycle arrest in p53-competent colorectal cancer cells. experimental and therapeutic medicine. 2010;1(2):369-75.
29. Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A, et al. Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. International journal of oncology. 2004;25(4):857-66.
30. Thabet NA, El-Khouly D, Sayed-Ahmed MM, Omran MM. Thymoquinone chemosensitizes human colorectal cancer cells to imatinib via uptake/efflux genes modulation. Clinical and Experimental Pharmacology and Physiology. 2021;48(6):911-20.

31. Jrah-Harzallah H, Ben-Hadj-Khalifa S, Almawi WY, Maaloul A, Houas Z, Mahjoub T. Effect of thymoquinone on 1, 2-dimethyl-hydrazine-induced oxidative stress during initiation and promotion of colon carcinogenesis. European Journal of Cancer. 2013;49(5):1127-35.
32. Jelski W, Mroczko B. Biochemical Markers of Colorectal Cancer–Present and Future. Cancer Management and Research. 2020;12:4789.
33. Gao Y, Wang J, Zhou Y, Sheng S, Qian SY, Huo X. Evaluation of serum CEA, CA19-9, CA72-4, CA125 and ferritin as diagnostic markers and factors of clinical parameters for colorectal cancer. Scientific reports. 2018;8(1):1-9.
34. Mousa HFM, Abd-El-Fatah NK, Darwish OA-H, Shehata SF, Fadel SH. Effect of Nigella sativa seed administration on prevention of febrile neutropenia during chemotherapy among children with brain tumors. Child's Nervous System. 2017;33(5):793-800.
35. Bernardini S, Tiezzi A, Laghezza Masci V, Ovidi E. Natural products for human health: an historical overview of the drug discovery approaches. Natural product research. 2018;32(16):1926-50.
36. Atanasov AG, Waltenberger B, Pferschy-Wenzig E-M, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology advances. 2015;33(8):1582-614.
37. Zhang Q-Y, Wang F-X, Jia K-K, Kong L-D. Natural product interventions for chemotherapy and radiotherapy-induced side effects. Frontiers in pharmacology. 2018;9:1253.
38. Ansary J, Giampieri F, Forbes-Hernandez TY, Regolo L, Quinzi D, Gracia Villar S, et al. Nutritional Value and Preventive Role of Nigella sativa L. and Its Main Component Thymoquinone in Cancer: An Evidenced-Based Review of Preclinical and Clinical Studies. Molecules. 2021;26(8):2108.
39. Bourgou S, Pichette A, Marzouk B, Legault J. Antioxidant, anti-inflammatory, anticancer and antibacterial activities of extracts from Nigella sativa (black cumin) plant parts. Journal of Food Biochemistry. 2012;36(5):539-46.
40. Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H. Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. Journal of pharmacopuncture. 2017;20(3):179.
41. Randhawa MA, Alghamdi MS. Anticancer activity of Nigella sativa (black seed)—a review. The American journal of Chinese medicine. 2011;39(06):1075-91.
42. Khan A, Chen H, Tania M, Zhang DZ. Anticancer activities of Nigella sativa (black cumin). African Journal of Traditional, Complementary and Alternative Medicines. 2011;8(5S).
43. Lakemeyer L, Sander S, Wittau M, Henne-Bruns D, Kornmann M, Lemke J. Diagnostic and Prognostic Value of CEA and CA19-9 in Colorectal Cancer. Diseases. 2021;9(1):21.
44. Sarman H, Bayram R, Benek S. Anticancer drugs with chemotherapeutic interactions with thymoquinone in osteosarcoma cells. Eur Rev Med Pharmacol Sci. 2016;20(7):1263-70.
45. Korak T, Ergül E, Sazci A. Nigella sativa and cancer: A review focusing on breast cancer, inhibition of metastasis and enhancement of natural killer cell cytotoxicity. Current pharmaceutical biotechnology. 2020;21(12):1176-85.