# Efficacy of Oral Nano-Silymarin Formulation in the Prevention of Bone Marrow Suppression Induced by XELOX or m-FOLFOX6 Regimens in Metastatic Colorectal Cancer

**Hedyieh Karbasforooshan[1], Hossein Rahimi[2], Omid Arasteh[1], Abolghasem Allahyari[3], Mehdi Varmaghani[4,5], Sepideh Elyasi[1*]**

*[1]Department of Clinical Pharmacy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.*

*[2]Department of Internal Medicine, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.*

*[3]Department of Hematology-Oncology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*

*[4]Social Determinants of Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*

*[5]Department of Management Sciences and Health Economics, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.*

***Received:2025-02-28, Revised:2025-08-27, Accepted: 2025-09-02., Published:2025-09-30***

**Abstract**

***Background:*** Bone marrow suppression (BMS) is one of the main complications of chemotherapeutic agents. Given its anti-inflammatory, anti-oxidant, and anti-apoptotic properties, silymarin, a flavonoid derived from Silybum marianum, has been used against chemotherapy-induced BMS. The purpose of this research was to elucidate the advantages of oral nano-silymarin as a supplement to XELOX (oxaliplatin and capecitabine) or m-FOLFOX6 (folinic acid, fluorouracil, and oxaliplatin) regimen in relieving BMS in patients with metastatic colorectal cancer.

***Methods:*** A clinical trial was conducted on 60 individuals through a randomized, triple-blinded, placebo-controlled design. The participants received 70 mg capsules with 15% silymarin nano micelles or placebo capsules, twice daily after meals for the duration of six courses of XELOX or m-FOLFOX6, starting from the first day of treatment. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5 was applied to evaluate the grade of BMS following the third and sixth chemotherapy cycles.

***Results:*** The nano-silymarin group had significantly lower median CTCAE scores for both thrombocytopenia and neutropenia at the end of the third course ($P<0.001$). However, the difference remained significant only for neutropenia at the end of the sixth course ($P<0.001$). On the other hand, nano-silymarin did not have protective effects against anemia. Moreover, based on the frequency of CTCAE grading scores, the differences remained significant until the end of the study for thrombocytopenia and neutropenia, but just until the end of the third course for anemia.

***Conclusion:*** Nano-silymarin may display preventive effects against chemotherapy-induced bone marrow suppression, particularly neutropenia and thrombocytopenia in patients with metastatic colorectal cancer. However, further studies with larger sample sizes are required.

J Pharm Care 2025; 13(3): 150-161.

**Keywords:** Nano-Silymarin; Metastatic Colorectal Cancer; XELOX; m-FOLFOX6; Bone Marrow Suppression

## Introduction

Cancer is the leading cause of death, accounting for nearly 10 million deaths in 2020 (1). Colorectal cancer (CRC) accounts for 10% of all cancer diagnoses and 9% of cancer-related deaths worldwide (2). CRC is the third most common cancer in men and the fourth most prevalent in women in Iran (3). It is the leading cause of cancer-related mortality in men under the age of 50 (2). Approximately one-third of patients diagnosed with CRC

*** Corresponding Author:** Sepideh Elyasi
Address: Department of Clinical Pharmacy, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran, P.O. Box 91775-1365.
Email: elyasis@mums.ac.ir

will develop metastases either at the time of diagnosis or during follow-up (4). The cornerstone of treatment in CRC patients is systemic therapy, as it can significantly improve long-term survival. Despite the availability of potent cytotoxic agents, metastatic (mCRC) has a poor five-year survival rate, ranging from 10% to 30%. The FOLFOX (comprising folinic acid, fluorouracil, and oxaliplatin) and XELOX (consisting of oxaliplatin and capecitabine) have been widely used as first-line treatment for mCRC (5). A significant proportion of cancer patients undergoing chemotherapy experience a range of side effects, from mild to severe. It is well established that specific chemotherapeutic agents can induce bone marrow suppression (BMS), leading to neutropenia, anemia, and thrombocytopenia. Consequently, treatment discontinuation or the use of potentially sub-therapeutic doses is performed to mitigate myelosuppression, lower the incidence of infections, and prevent febrile neutropenia. Thus, the development of non-toxic alternative therapeutic options in cancer care is of critical importance (6).

In recent years, herbal medicines have been introduced as a complementary approach to reduce the chemotherapy-induced adverse effects. Milk thistle (Silybum marianum) has been used as a medicinal plant for many years. It contains silymarin, which consists of seven flavonolignans along with fatty acids and other polyphenolic components. Silibinin, the primary bioactive ingredient, accounts for nearly 60–70% of the total silymarin content. Silymarin is primarily derived from the seeds of milk thistle and has been employed as a natural herbal therapy for several conditions, such as liver diseases and cirrhosis. Studies have revealed that silymarin modulates multiple cellular pathways, exerting antioxidant effects and regulating inflammation and apoptosis (7). Silymarin exerts its anti-inflammatory effects by downregulation of different inflammatory mediators, such as nuclear factor kappa B (NF-κB), tumor necrosis factor (TNF)-α, interleukin (IL)-2, IL-6, IL-1B, interferon (IFN)-γ, prostaglandin (PG) E2, PGF2, and cyclooxygenase (COX)-2, as well as reduced nitric oxide generation (8). Furthermore, silymarin has also been shown to neutralize reactive oxygen species (ROS), reduce lipid peroxidation, and enhance antioxidant enzyme activity (9). Clinical studies proved the safety of silymarin for human consumption at therapeutic dosages, such as 700 mg three times daily for 24 weeks. The most common reported side effects are gastrointestinal symptoms, followed by headaches, gastroenteritis, and dermatological manifestations (10). Although rare, allergic reactions—typically presenting as skin rashes, swelling, or itching—have been documented following silymarin administration, particularly in individuals with a history of plant allergies (11). Silymarin has poor oral bioavailability due to its lipophilic nature, high metabolism, and fast excretion. Actually, the major challenge in producing silymarin formulations is their poor aqueous solubility. It has been revealed that almost 50% of silymarin is absorbed via the gastrointestinal tract (12). As a drug delivery system, micellar nanoparticles have attracted attention because of their ability to elevate drug loading capacity, protect against drug degradation, and boost tissue penetration (13). They enhance the solubility of silymarin in water by 3,000 times, and exhibit stability in the stomach for a minimum of three hours, reaching the small intestine (14). One study showed that the nano-micelle silymarin absorption in various segments of the intestine was significantly higher than that of free silymarin in rats (15). Another study documented that encapsulating silymarin in micelles can effectively enhance its anticancer properties through colon cancer cells apoptosis (16). Therefore, nano-silymarin utilization in clinical research may improve its benefits for oral administration (17). In this study, we employed micelle nanoparticles to boost drug delivery efficacy and improve both the stability and solubility.

Given the lack of human clinical trials evaluating the potential of silymarin to attenuate bone marrow suppression, the present investigation aimed to assess the effects of an oral nano-silymarin formulation on preventing bone marrow suppression in cancer patients undergoing XELOX or mFOLFOX6 regimens. This study, to the best of our knowledge, is the first triple-blinded, randomized clinical trial in this field.

## Methods

### *Study design*

This study was a triple-blind, balanced, randomized, and placebo-controlled clinical trial. Patient selection was conducted from January 2021 to July 2023 at an oncology outpatient center in Mashhad, Iran. The trial was registered on the Iranian Registry of Clinical Trials (IRCT20200408046990N5).

It is worth mentioning that some parts of the methods used in this study are consistent with those described in Karbasforooshan et al.'s study, as we present another secondary outcome of this clinical trial (18).

### *Eligibility criteria*

Individuals aged 18 to 70 years with metastatic colorectal cancer receiving either the XELOX or m-FOLFOX6 chemotherapy regimens were included. Regarding the selection of the mCRC population for this study, several studies have investigated the effectiveness of silymarin in colorectal cancer (19-21). As the primary outcome of our RCT was evaluating nano-silymarin efficacy as an adjuvant treatment in cancer patients, we selected this population.

The liver function test (alanine transaminase (ALT) and aspartate transaminase (AST) levels) should not exceed

five times the upper limit of normal (ULN). Furthermore, total bilirubin levels should not exceed two times the ULN. Moreover, creatinine levels should not exceed 1.5 mg/dL. Written consent was required for participation in the study. Exclusion criteria included: incapacitation to swallow capsules, pregnancy or breastfeeding, a history of allergy to silymarin, heart failure, autoimmune disorders, acquired or drug-induced immunodeficiency (excluding those due to chemotherapy), multiple primary cancers, and concomitant regular use including dietary supplements (vitamin C: >500 mg/day, vitamin E: >400 IU/day, beta-carotene: >5,000 IU/day, selenium: >100 mcg/day, N-acetylcysteine (NAC): >600 mg/day, alpha-lipoic acid: >300 mg/day, coenzyme Q10 >100 mg/day), antioxidant-rich herbal supplements (green tea extract: >500 mg/day, resveratrol supplements: >100 mg/day, grape seed extract: >100 mg/day, pine bark extract: >100 mg/day) within two weeks prior to study initiation.

Regular use was defined as ≥3 times per week for the past month. Exceptions were allowed for standard multi-vitamins that contain antioxidants at the recommended dietary allowance (RDA) levels. Dietary origin of antioxidants through normal food consumption was also allowed.

The exclusion criteria included any deterioration in patients' condition during treatment, requiring a therapy adjustment, and the presence of intolerable complications as defined by the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE version).

***Randomization and blinding***

Patients were assigned to either the nano-silymarin group or the placebo group using a randomization list generated from the website "randomization.com" in a 1:1 ratio. A block randomization method involving four patients was employed to ensure an impartial distribution of the qualified patients between the control and intervention groups. Both the nano-silymarin and placebo capsules were filled in uniform containers produced by Exir Nano Sina Company, with the silymarin and placebo labeled as A and B, respectively. At the start of the study and again after four weeks, patients obtained two boxes of the medication, each including 120 soft gels (each containing 70 mg) of either silymarin nano-micelles or placebo, supplying an adequate reservoir for eight weeks. Both the oncologist and the clinical pharmacist evaluated the patients throughout the course of treatment without awareness of their group placement. Throughout the study, the statistician remained unaware of the group allocations.

***Intervention***

Two groups of patients were randomly assigned; one group received nano-silymarin, while the other group received a placebo. The treatment group was administered nano-micelle capsules (70 mg) twice daily after meals (SinaLiveR; Exir Nano, Pharmaceutical Company, Tehran, Iran, registered code: 6231566784211002) for six courses of either the XELOX or modified FOLFOX6 protocols, starting on the first day of their regimen. The placebo capsules, prepared by the same company using a similar process, contained all the same components except for silymarin. We chose this daily dosing of nano-silymarin based on the manufacturer's recommendation for use in other indications, such as liver diseases (22). Adherence to the treatment was measured based on whether patients consumed more than 80% of the administered capsules. The modified FOLFOX6 regimen involves an infusion of oxaliplatin at a dose of 85 mg/m2, leucovorin at 400 mg/m2, and a bolus of fluorouracil at 400 mg/m2 on the first day, then a fluorouracil infusion of 2400 mg/m2 over 46-48 hours, repeated every two weeks. The XELOX regimen involves an infusion of oxaliplatin at 130 mg/m2 on day 1, followed by oral capecitabine at 1,000 mg/m2 taken twice daily from the evening of day 1 until the morning of day 15, with a subsequent 7-day treatment-free interval, all within a 3-week cycle (23).

***Study endpoints***

Before the intervention, measurements of hemoglobin levels, platelet counts, white blood cell counts (WBC), and absolute neutrophil counts (ANC) were taken. These variables were remeasured after the third and sixth courses of treatment. To specify the incidence of bone marrow suppression, oncologists reviewed patient data using the CTCAE version 5 score at the end of each treatment course. The CTCAE grades complications based on their severity, using a 5-point scale (Grade 1 to Grade 5) (24). Throughout the study, patients were closely monitored for treatment compliance and any adverse drug reactions.

***Sample size calculation***

This study was proposed as a pilot study. It is the first clinical research to investigate the impact of nano-silymarin in the prevention of chemotherapy-induced bone marrow suppression. Following the guidelines provided by Whitehead et al., a main trial designed to attain 90% power with a two-sided significance level of 5% would require pilot trial sample sizes of 75, 25, 15, and 10 for normalized effect sizes classified as minimal (≤0.1), small (0.2), medium (0.5), or large (0.8), respectively (25). As a result, the normalized effect size for nano-silymarin in this investigation was anticipated to be in the small to medium range. Considering a study power of 90% ($\beta$=0.20) and $\alpha$ error of 5%, a sample size of 15-25 is adequate, leading to defining a sample size of 25 subjects for each arm. According to the potential dropouts, another five patients

were added to each arm, resulting in a total sample size of 30 patients per study arm.

### *Statistical analysis*

Data analysis was performed using SPSS software, version 27.0 (SPSS INC/IBM Corp., Chicago, IL, USA). The mean, accompanied by the standard deviation, was used to represent continuous variables. On the other hand, variables with non-normal distribution were reported through the median and interquartile ranges. Nominal variables were illustrated as numbers (percentages). To assess the normality of the distributions of the variables, the Kolmogorov-Smirnov test was utilized. The quantitative variables between the two groups were compared using an independent sample t-test. Fisher's exact test was implemented for qualitative variables. The Mann-Whitney test was used in the case of non-normal distribution of quantitative data. For multiple time-dependent comparisons within each group, the Friedman test was used. The Wilcoxon signed-rank test was also used as a post-hoc test for the Friedman test for multiple related groups. Statistical significance was defined as a p-value of less than 0.05. To account for multiple comparisons, a Bonferroni correction was applied to the p-values obtained from the Wilcoxon signed-rank tests. This correction adjusts the significance level by dividing the standard alpha level (0.05) by the number of comparisons being made. As three comparisons were made in this study, a p-value of less than 0.0167 (0.05/3) was considered statistically significant.

### *Ethical considerations*

The study protocol was approved by the local Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.REC.1399.503). This research was in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research. All participants signed the written consent forms.

## Results

### *Baseline characteristics*

A total of 68 patients were enrolled in the study, with 60 patients ultimately analyzed (Figure 1).

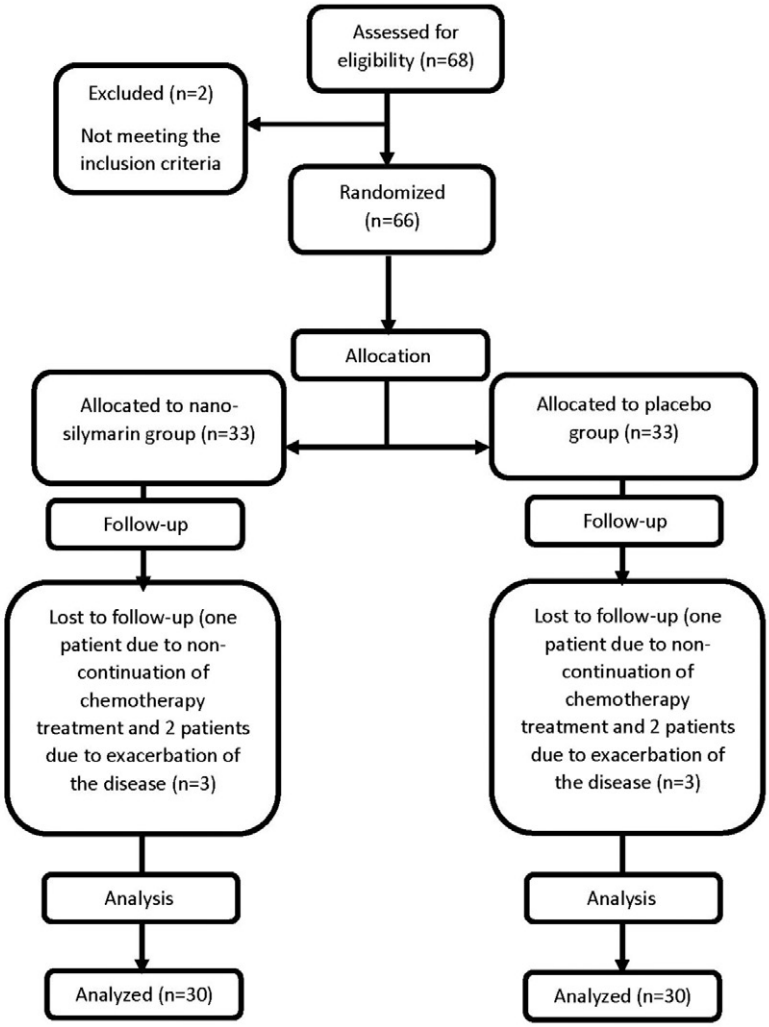


**Figure 1. Flow diagram of the study design**

The mean age of the patients was 50.3±11.17 years, and 50% of the patients were female. Seventy percent of the patients were prescribed the FOLFOX regimen, while 30% of the patients were on the XELOX regimen. Apart from the metastatic site, there were no significant variations in baseline demographic and biochemical parameters between the two groups (Table 1).

**Table 1. Patient characteristics and biochemical parameters**

| | | Nano-silymarin group (n=30) | Placebo group (n=30) | P- value |
|---|---|---|---|---|
| **Weight (kg) mean ± SD** | | 64.86 ±10.51 | 68.43 ±11.70 | 0.219[a] |
| **Height (cm) mean ± SD** | | 164.46 ±10.51 | 168 ±8.42 | 0.066[a] |
| **Body surface area ($m^2$) median (range)** | | 1.75 (1.59-1.81) | 1.78 (1.65-1.94) | 0.141[b] |
| **Sex (n (%))** | **Female** | 14 (46.7) | 16 (53.3) | 0.606[c] |
| | **Male** | 16 (53.3) | 14 (46.7) | |
| **Comorbidity disease (n (%))** | **Diabetes** | 11 (36.7) | 5 (16.7) | 0.08[c] |
| | **Hypertension** | 5 (16.7) | 6 (20) | 0.739[c] |
| | **Cardiovascular diseases** | 0 (0) | 1 (3.3) | 1[c] |
| | **Hypothyroidism** | 0 (0) | 1 (3.3) | 1[c] |
| | **Hyperlipidemia** | 0 (0) | 1 (3.3) | 1[c] |
| **Concurrent medicines (n (%))** | **Antidiabetic** | 11 (36.7) | 5 (16.7) | 0.08[c] |
| | **Antihypertensive** | 5 (16.7) | 6 (20) | 0.739[c] |
| | **Cardiovascular medications** | 0 (0) | 1 (3.3) | 1[c] |
| | **Hypothyroidism medications** | 0 (0) | 1 (3.3) | 1[c] |
| | **Lipid-lowering medications** | 0 (0) | 1 (3.3) | 1[c] |
| **ECOG (n (%))** | **Zero** | 16 (53.3) | 16 (53.3) | 1[c] |
| | **One** | 14 (46.7) | 14 (46.7) | |
| **Chemotherapy regimen (n, (%))** | **FOLFOX** | 24 (80) | 18 (60) | 0.091[c] |
| | **XELOX** | 6 (20) | 12 (40) | |
| **Metastasis site (n (%))** | **Liver** | 20 (66.7) | 8 (26.7) | 0.009[c] |
| | **Lung** | 0 (0) | 5 (16.7) | |
| | **Peritoneum** | 8 (26.7) | 8 (26.7) | |
| | **Lymph** | 0 (0) | 3 (10) | |
| | **Liver, Lung** | 2 (6.7) | 5 (16.7) | |
| | **Liver, Bone** | 0 (0) | 1 (3.3) | |
| **WBC (× $10^9$/L) median (range)** | | 5.7 (4.475-7.7) | 4.85 (4.575-6.1) | 0.208[b] |
| **ANC (× $10^9$/L) median (range)** | | 3.42 (2.625-4.53) | 2.91 (2.745-3.66) | 0.450[b] |
| **Hemoglobin (g/L) median (range)** | | 11.6 (9.45-13.5) | 12.5 (11.37-13) | 0.149[b] |
| **Platelet (× $10^9$/L) median (range)** | | 198 (173.5-257.5) | 197.5 (163-231) | 0.355[b] |

[a] Independent-sample T test, [b] Mann-Whitney test, [c] Chi-squared test. SD: Standard Deviation, ECOG: Eastern Cooperative Oncology Group, WBC: White Blood Count; ANC: Absolute Neutrophil Count

Evaluation of nano-silymarin efficacy in the prevention of chemotherapy-induced bone marrow suppression

The difference between the two groups in red blood cell (RBC) count and hemoglobin serum level was not significant at either the third or sixth weeks. However, there was a statistically significant difference between the groups in terms of WBC, ANC, and platelets after three courses of therapy (P<0.05). After six courses, the difference in WBC and ANC remained significant, but the platelet count was no longer statistically significant (Table 2).

**Table 2. Patient hematological parameters after three and six courses of treatment**

| Hematological Parameters | Nano-silymarin group (n=30) | Placebo group (n=30) | P-value |
|---|---|---|---|
| **After three courses of treatment** | | | |
| **WBC (× 109/L, median (range])** | 5.375 (4.3-7.5] | 3.75 (3.175-4.75] | <0.001[a] |
| **ANC (× $10^9$/L, median (range])** | 3.225 (2.616-4.5] | 2.15 (1.845-2.85] | <0.001[a] |
| **Hemoglobin (g/L, median (range])** | 11 (9.8-12.8] | 12 (11-12.55] | 0.343[a] |
| **Platelet (× $10^9$/L, median (range])** | 156500 (138000-183500] | 143000 (114500-163500] | 0.047[a] |
| **After six courses of treatment** | | | |
| **WBC (× $10^9$/L, median (range])** | 5.250 (4.1-7.1] | 3.45 (3.1-4.85] | 0.002[a] |
| **ANC (× $10^9$/L, median (range])** | 3.1 (2.595-4.26] | 2.040 (1.8-2.895] | <0.001[a] |
| **Hemoglobin (g/L, median (range])** | 10.85 (9.8-12] | 12 (10.3-12.15] | 0.139[a] |
| **Platelet (× $10^9$/L, median (range])** | 72 (68-151.5] | 125 (76.75-145] | 0.141[a] |

WBC: White Blood Count; ANC: Absolute Neutrophil Count, [a]Mann-Whitney test

Throughout the treatment, consistent with WBC and ANC counts, the median National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) neutropenia scores were considerably higher in the silymarin group. The scores remained zero in the nano-silymarin group, but reached one in the placebo group (P<0.05) (Table 3). Moreover, comparing the frequency of each CTCAE neuropathy grade between the two groups, approximately 93.3% of patients in the treatment group still had a zero neutropenia score following three courses of chemotherapy. However, 43.3% had a zero neutropenia score in the placebo group. At the end of the sixth course, 93.3% of patients still did not have neutropenia in the nano-silymarin group, but no changes were observed in the placebo group.

**Table 3. NCI-CTCAE chemotherapy-induced adverse effects scores of the silymarin and placebo groups**

| Adverse Effects | | Nano-silymarin group (n=30) Median (quartile range) | Placebo group (n=30) Median (quartile range) | P-value[a] |
|---|---|---|---|---|
| **Neutropenia** | **at the beginning** | 0 (0-0) | 0 (0-0) | - |
| | **at the end of course 3** | 0 (0-0) | 1 (0-1) | <0.001 |
| | **at the end of course 6** | 0 (0-0) | 1 (0-1) | <0.001 |
| **Thrombocytopenia** | **at the beginning** | 0 (0-0) | 0 (0-0) | - |
| | **at the end of course 3** | 1 (0-1) | 1 (1-1.25) | <0.001 |
| | **at the end of course 6** | 2 (0-2) | 1 (0-1) | 0.191 |
| **Anemia** | **at the beginning** | 1 (0-2) | 1 (0-1) | 0.07 |
| | **at the end of course 3** | 1 (0-2) | 1 (1-1) | 0.842 |
| | **at the end of course 6** | 1 (0-2) | 1 (1-1) | 0.974 |

NCI-CTCAE: National Cancer Institute-Common Terminology Criteria for Adverse Events, [a]Mann–Whitney test

No participant exhibited grade 5 in both groups. It should be noted that, in the nano-silymarin group, two patients developed grade 4 neutropenia after three courses. Still, their neutropenia resolved to grade 1 by the end of the sixth course. In confirmation of previous findings, the difference in the frequency of these scales between the two groups was also significant after 3 and 6 courses (P<0.001) (Table 4).

**Table 4. Comparison of NCI-CTCAE v.5 neuropathy scores between the two groups during the study period**

| Adverse reaction | Time point | Group | Grade 0 n (%) | Grade 1 n (%) | Grade 2 n (%) | Grade 3 n (%) | Grade 4 n (%) | Grade 5 n (%) | P-value[a] |
|---|---|---|---|---|---|---|---|---|---|
| Neutropenia | Beginning | Nano-silymarin | 27(90) | 2(6.7) | 0(0) | 1(3.3) | 0(0) | 0(0) | 0.431 |
| | | Placebo | 26(86.7) | 4(13.3) | 0(0) | 0(0) | 0(0) | 0(0) | |
| | Course 3 | Nano-silymarin | 28(93.3) | 0(0) | 0(0) | 0(0) | 2(6.7) | 0(0) | <0.001 |
| | | Placebo | 13(43.3) | 13(43.3) | 4(13.3) | 0(0) | 0(0) | 0(0) | |
| | Course 6 | Nano-silymarin | 28(93.3) | 2(6.7) | 0(0) | 0(0) | 0(0) | 0(0) | <0.001 |
| | | Placebo | 13(43.3) | 13(43.3) | 4(13.3) | 0(0) | 0(0) | 0(0) | |
| Thrombocytopenia | Beginning | Nano-silymarin | 30(100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0.117 |
| | | Placebo | 26(86.7) | 2(6.7) | 2(6.7) | 0(0) | 0(0) | 0(0) | |
| | Course 3 | Nano-silymarin | 21(70) | 9(30) | 0(0) | 0(0) | 0(0) | 0(0) | 0.001 |
| | | Placebo | 8(26.67) | 17(56.7) | 5(16.7) | 0(0) | 0(0) | 0(0) | |
| | Course 6 | Nano-silymarin | 10(33.3) | 2(6.7) | 16(53.33) | 2(6.7) | 0(0) | 0(0) | <0.001 |
| | | Placebo | 6(20) | 17(56.7) | 6(20) | 1(3.3) | 0(0) | 0(0) | |
| Anemia | Beginning | Nano-silymarin | 11(36.7) | 8(26.7) | 11(36.7) | 0(0) | 0(0) | 0(0) | 0.004 |
| | | Placebo | 13(43.3) | 16(53.3) | 1(3.3) | 0(0) | 0(0) | 0(0) | |
| | Course 3 | Nano-silymarin | 12(40) | 9(30) | 9(30) | 0(0) | 0(0) | 0(0) | <0.001 |
| | | Placebo | 4(13.3) | 25(83.3) | 1(3.3) | 0(0) | 0(0) | 0(0) | |
| | Course 6 | Nano-silymarin | 8(26.7) | 13(43.3) | 9(30) | 0(0) | 0(0) | 0(0) | 0.113 |
| | | Placebo | 4(13.3) | 21(70) | 5(16.7) | 0(0) | 0(0) | 0(0) | |

[a]Chi-squared test

The median CTCAE thrombocytopenia score was significantly lower in the silymarin group at the end of the third course (P<0.001), but not at the end of the sixth course (p-value = 0.191) (Table 3). However, regarding the frequency of thrombocytopenia CTCAE scales, a significant difference was observed between the two groups at the end of both the third (P=0.001) and sixth (P<0.001) courses. Following three courses of chemotherapy, 70% of patients in the treatment group still had a zero thrombocytopenia score. However, 26.7% had a zero thrombocytopenia score in the placebo group. At the end of the sixth course, 33.3% of patients did not have thrombocytopenia in the nano-silymarin group, and 20% of patients did not have thrombocytopenia in the placebo group. No individual exhibited grade 4 and 5 in both groups (Table 4).

The median CTCAE anemia score remained one in both groups throughout therapy without any significant difference between the two groups (Table 3). But comparing the frequency of CTCAE anemia scales between the two groups, there was only a significant difference at the end of the third course (P<0.001). Following three courses of chemotherapy, 40% of patients in the treatment group still had a zero anemia score. However, 13.3% had a zero anemia score in the placebo group. At the end of the sixth course, 26.7% of patients did not have anemia in the nano-silymarin group, but no changes were observed in the placebo group. No participant exhibited grades 4 and 5 in both groups (Table 4).

Performing intra-group assessment during the study in the silymarin and placebo group, the Friedman test result showed a significant increase in anemia CTCAE scores (p-value < 0.001), thrombocytopenia (p-value < 0.001), and neutropenia in the placebo group (p-value <0.001). The Wilcoxon post hoc test was performed for them.

According to the results, in the placebo group, the scores of anemia and thrombocytopenia at the end of courses three and six had a significant increase compared to the beginning of the study (p-value<0.005). However, there was no significant increase at the end of course six compared to course three (p-value > 0.0167). The same finding was recorded for the neutropenia (p-value < 0.001) (Table 5).

**Table 5. Comparison of the median scores of CTCAE during chemotherapy in nano-silymarin and placebo groups**

| | Beginning vs. course 3 | Beginning vs. course 6[b] | Course 3 vs. course 6[b] | P-value[a] |
|---|---|---|---|---|
| **Nano-silymarin group** | | | | |
| Neutropenia | - | - | - | 0.368 |
| Thrombocytopenia | 0.003 | <0.001 | <0.001 | <0.001 |
| Anemia | - | - | - | 0.236 |
| **Placebo group** | | | | |
| Neutropenia | <0.001 | <0.001 | 1 | <0.001 |
| Thrombocytopenia | <0.001 | <0.001 | 0.166 | <0.001 |
| Anemia | 0.003 | <0.001 | 0.046 | <0.001 |

[a]Friedman test, [b]Wilcoxon signed-rank test, CTCAE: Common Terminology Criteria for Adverse Events

In the treatment group, only a significant increase was found regarding the thrombocytopenia (P<0.001), but not anemia (P=0.236) and neutropenia (P=0.368). Considering Post hoc analysis with Wilcoxon signed-rank test results, the score increased significantly at the end of course 6 compared to the beginning of the study and course 3 (p-value = <0.001), and also at the end of course 3 compared to the beginning of the study (p-value = 0.003) (Table 5).

### *Patient safety*

The patients experienced no documented adverse reaction from either the placebo or the nano-silymarin capsules.

## Discussion

This clinical trial examined the effectiveness of oral nano-silymarin in the prevention of chemotherapy-induced bone marrow suppression following XELOX and m-FOLFOX6 regimens.

Due to its anti-inflammatory properties and its ability to regulate oxidative stress and apoptosis, silymarin is widely recognized for its various therapeutic benefits in managing diseases, including chemotherapy-related complications (7). In therapeutic modalities, polymeric micelles are employed due to their ability to enhance solubility (12). Several studies have demonstrated that nano-formulations can elevate the bioavailability of silymarin. Therefore, it can be inferred that nano-silymarin improves clinical outcomes by attaining higher plasma concentrations of the active ingredient (26).

Chemotherapy can lead to toxic effects. FOLFOX and XELOX regimens are associated with an increased risk of bone marrow suppression (27). Chemotherapy-induced neutropenia (CIN) is a significant dose-limiting toxicity in cancer therapy. Neutropenia predisposes patients to potentially lethal adverse effects, such as febrile neutropenia, and increases antibiotic utilization, hospitalization, and mortality (28). The risk of febrile neutropenia is minimal with capecitabine (29). It has been found that Grades 3 and 4 neutropenia develops nearly seven times more often in patients receiving bolus doses of fluorouracil compared to those who were not treated with this medication (30). Moderate to severe neutropenia may be induced by oxaliplatin administration, with approximately 4% of subjects developing neutropenic fever (31). In mice treated with oxaliplatin, white blood cell counts decreased in a dose-dependent manner (32). It is noteworthy that investigations have reported the incidence of grade 3 or higher neutropenia during XELOX therapy to range from 8.6% to 12.6%. Prophylactic administration of granulocyte colony-stimulating factors (G-CSFs) and chemotherapy dose reduction are common methods to relieve CIN (28). G-CSF administration has been shown to improve patient outcomes by reducing the incidence of CIN. Nevertheless, their economic burden and the bone pain-induced disturbance they cause remain serious challenges (27). Chemotherapy-induced thrombocytopenia may lead to life-threatening bleeding. To date, no standardized guidelines exist for the management of chemotherapy-induced thrombocytopenia. In severe cases, chemotherapy dose adjustment is required to attenuate the risk of bleeding or the need for platelet transfusions. However, such an adjustment may compromise therapeutic efficacy. Although platelet transfusions can temporarily control serious thrombocytopenia, they are associated

with several problems, such as allogeneic immunity, transfusion reactions, and the potential transmission of infectious pathogens (33). In addition, oxaliplatin-induced thrombocytopenia may be related to myelosuppression, immune-mediated responses, and splenomegaly resulting from hepatic sinusoidal obstruction syndrome (SOS) (34). Chemotherapy typically interferes with regular hematopoiesis. Oxaliplatin can increase platelet clearance with mononuclear phagocytes through an immune mechanism involving drug-dependent antibodies (35). This status is known as drug-induced immune thrombocytopenia (DITP). Among all chemotherapeutic agents, oxaliplatin remains the most common cause of DITP. The main sign of oxaliplatin-induced immune thrombocytopenia (OIIT) is the sudden onset of severe thrombocytopenia (33). Although oxaliplatin-induced thrombocytopenia is rare, Grade 3 and 4 thrombocytopenia occurs in approximately 3-4% of patients (31). Chemotherapy-induced anemia (CIA) is another significant complication of cancer treatment. Alongside increased fatigue and a decreased quality of life, CIA can disrupt or delay therapeutic processes.

Management of CIA includes iron supplementation, erythropoietin-stimulating agents (ESAs), and transfusions of packed red blood cells. However, a considerable proportion of patients do not receive treatment due to concerns about tumor progression and the potential adverse effects associated with ESAs (36). Red blood cells were negatively affected by oxaliplatin in an in vitro study. However, anemia usually presents in a mild form, with most patients experiencing grades 1 to 3 anemia (32), while only a small proportion presents with grade 4 or 5. Several cases of oxaliplatin-induced hemolytic anemia have been reported. In addition, some animal studies revealed oxaliplatin-induced macrocytic anemia (37). Rare reports of capecitabine-induced hemolytic anemia via a non-immunological mechanism have also been documented (38).

Fluorouracil is exclusively detrimental to rapidly proliferating tissues, such as the bone marrow. Grade 3-4 hematological toxicities are more prevalent with bolus administration of fluorouracil (30). A dosage of 150 mg/kg has been shown to impair the erythropoietin production, and significantly lower the number of mature erythroid cells, red blood cells, and erythropoietic precursors in the bone marrow. Fluorouracil-induced damage prohibits the entry of these cells into the cell cycle, effectively preventing their progression. Entry into the cell cycle by hematopoietic stem cells is, in part, dependent on the regulation of hematopoietic cytokines. Interferon-gamma (IFN-γ) levels of which are increased by fluorouracil, can induce immature apoptosis of erythroid progenitor cells. Furthermore, fluorouracil has been linked to hemolytic anemia. An investigation proved a remarkable reduction in red blood cell counts, hemoglobin levels, and hematocrit after 10 days of intravenous fluorouracil administration in rats. In addition, the cellular components of the bone marrow and their proliferative capacity were markedly reduced (39).

Currently, no studies have reported the protective properties of silymarin against neutropenia, thrombocytopenia, and anemia due to FOLFOX or XELOX. However, it has been found that cisplatin-induced anemia, thrombocytopenia, and bone marrow suppression in rats were alleviated by oral silymarin administration at a dose of 100 mg/kg for 10 days. Silymarin appears to exert its bone marrow-protective effects through the improvement of the myeloid maturation index. The amelioration in hematological parameters was attributed to this mechanism (40). In another study, anemia, thrombocytopenia, leukopenia, elevated inflammatory cytokines, and oxidative damage have been observed in mice infected with Mayaro virus. However, all these parameters were improved with silymarin treatment at a dosage of 100 mg/kg for 4 days (41). A study found that the daily administration of 100 mg/kg silymarin in pregnant rats with preeclampsia resulted in a significant increase in platelet counts and a reduction in necrosis factors TNF-α, IFN-γ, and IL-1β when compared to the placebo group (42).

Our study demonstrated the potential of nano-silymarin to decrease the incidence of neutropenia throughout the entire treatment period. The administration of G-CSFs is effective in improving neutropenia in patients. The number of patients requiring G-CSFs was two patients in both nano-silymarin and placebo groups, indicating no significant difference between the groups in this regard. Regarding the thrombocytopenia, also based on the frequency of the CTCAE grading scores, nano-silymarin was significantly effective throughout the study. However, comparing the median scores between the two groups, it was not able to preserve its effectiveness throughout the study period. It was only able to delay the onset of this complication. It is recommended to evaluate the efficacy of nano-silymarin in future studies on a larger sample size and for a longer duration.

In contrast, nano-silymarin showed no preventive effect on anemia based on the median CTCAE anemia scores only demonstrated significant efficacy after three courses of chemotherapy on the basis of the frequency of each anemia grade score. Given the delayed onset of anemia as a complication, the lack of effectiveness of silymarin in

preventing this complication may be related to this particular issue, and designing studies for a longer duration could be helpful for confirmation of this hypothesis. Consequently, this clinical study demonstrated that nano-silymarin can be an effective measure in preventing chemotherapy-induced bone marrow suppression, particularly against neutropenia and thrombocytopenia. The possibility that nano-silymarin may interfere with chemotherapy efficacy is another concern that must be addressed in this study. In this regard, the primary outcome of the current study was to investigate the effectiveness of nano-silymarin as an adjuvant treatment in patients with metastatic colorectal cancer. The evaluation was conducted based on the response evaluation criteria in solid tumors (RECIST) criteria and the carcinoembryonic antigen (CEA) serum level. The CEA serum level was not significantly different between the treatment and placebo groups, and some positive effects were seen based on the RECIST criteria. Actually, the risk of disease progression reduced with nano-silymarin use based on radiological assessment. These findings will be published in more detail shortly.

Our study has several limitations. First, the sample size was relatively small. Moreover, it would be feasible to evaluate the effectiveness of nano-silymarin in comparison with traditional formulations. Also, it would be prudent to perform complete blood count (CBC) testing at additional time points to gain a comprehensive understanding of hematologic recovery. In addition, further investigations are essential to determine the efficacy and safety of various doses of nano-silymarin in larger populations. Furthermore, it is recommended to assess biomarkers, such as serum concentrations of inflammatory mediators, to clarify the potential mechanisms of silymarin. Finally, future research may also evaluate the synergistic effects of nano-silymarin in combination with other antioxidants.

## Conclusion

Nano-silymarin may serve as an effective adjuvant in preventing chemotherapy-induced neutropenia and thrombocytopenia and postponing anemia. Further trials with larger sample sizes, different dosages, and longer durations of administration are recommended to determine the protective impact of nano-silymarin on bone marrow suppression, particularly anemia.

## Conflict of Interests

Dr. Mahmoud Reza Jaafari, is the founder of Exir Nano Sina Company, in which the studied medication was produced. Other authors have nothing to declare.

## Acknowledgments

This study was part of a research thesis for a Ph.D. degree and was supported by the Research Council of Mashhad University of Medical Sciences, Mashhad, Iran (No. 991041).

## References

1. Farashi E, Mahmoodpour A, Akbari AM, Sanaat Z, Sarbakhsh P, Chavoshi SH, et al. An Investigation of Insulin Resistance and Cachexia Relation in Patients with Metastatic Gastrointestinal Malignancies. J Pharm Care. 2022;10(4):195-204.
2. Dosunmu GT, Shergill A. Colorectal Cancer: Genetic Underpinning and Molecular Therapeutics for Precision Medicine. Genes (Basel). 2024;15(5):538.
3. Salehifar E, Gheibi Sh, Janbabaei Gh, Mousavi Kh. Adverse Effects of Chemotherapy Regimens Used in Colorectal Cancer Patients in a Referral Cancer Center in North of Iran, 2008-2014. J Pharm Care 2016; 4(1-2): 9-13.
4. Morris VK, Kennedy EB, Baxter NN, Benson III AB, Cercek A, Cho M, et al. Treatment of metastatic colorectal cancer: ASCO guideline. J Clin Oncol. 2023;41(3):678-700.
5. Underwood PW, Ruff SM, Pawlik TM. Update on targeted therapy and immunotherapy for metastatic colorectal cancer. Cells. 2024;13(3):245.
6. Ferro Y, Maurotti S, Tarsitano MG, Lodari O, Pujia R, Mazza E, et al. Therapeutic Fasting in Reducing Chemotherapy Side Effects in Cancer Patients: A Systematic Review and Meta-Analysis. Nutrients. 2023;15(12):2666.
7. Sharma U, Sahni PK, Sharma B, Gupta M, Kaur D, Mathkor DM, et al. Silymarin: a promising modulator of apoptosis and survival signaling in cancer. Discov Oncol. 2025;16(1):66.
8. Surai PF, Surai A, Earle-Payne K. Silymarin and Inflammation: Food for Thoughts. Antioxidants (Basel). 2024;13(1):98.
9. Gholami AH, Ansari H, Dadkhah A. Effect of Silybum Marianum on Reduction of Chemotherapy-Induced Peripheral Neurotoxicity with Cisplatin. Adv Biomed Res. 2024;13:21.

10. Soleimani V, Delghandi PS, Moallem SA, Karimi G. Safety and toxicity of silymarin, the major constituent of milk thistle extract: An updated review. Phytother Res. 2019;33(6):1627-38.

11. Dhande D, Dhok A, Anjankar A, Nagpure S. Silymarin as an Antioxidant Therapy in Chronic Liver Diseases: A Comprehensive Review. Cureus. 2024;16(8):e67083.

12. Mohebbati R, Momeni-Moghaddam MA, Asghari R, Abbasnezhad A, Bideskan AEZ, Salarbashi D, et al. The comparison of the effects of nano-silymarin and silymarin on high-fat diet-induced fatty liver of adult male rats. Avicenna J Phytomed. 2024;14(3):365-75.

13. Bose A, Roy Burman D, Sikdar B, Patra P. Nanomicelles: Types, properties and applications in drug delivery. IET nanobiotechnol. 2021;15(1):19-27.

14. Arab FL, Yousefi F, Jaafari MR, Rajabian A, Dana H, Tabasi N, et al. Evaluation of the immune-modulatory, anti-oxidant, proliferative, and anti-apoptotic effects of nano-silymarin on mesenchymal stem cells isolated from multiple sclerosis patients' adipose tissue sources. J Funct Foods. 2024;113:105958.

15. Mombeini M, Saki G, Khorsandi L, Bavarsad N. Effects of silymarin-loaded nanoparticles on HT-29 human colon cancer cells. Medicina (Kaunas). 2018;54(1):1.

16. Li X, Yuan Q, Huang Y, Zhou Y, Liu Y. Development of silymarin self-microemulsifying drug delivery system with enhanced oral bioavailability. Aaps Pharmscitech. 2010;11(2):672-8.

17. Di Costanzo A, Angelico R. Formulation strategies for enhancing the bioavailability of silymarin: the state of the art. Molecules. 2019;24(11):2155.

18. Karbasforooshan H, Rahimi H, Arasteh O, Allahyari A, Varmaghani M, Jannati M, et al. Evaluation of Oral Nano-Silymarin Formulation Efficacy in the Prevention of Hand-Foot Syndrome and Neuropathy Induced by XELOX or m-FOLFOX6 Regimens in Metastatic Colorectal Cancer: A Triple-Blinded, Randomized Clinical Trial. Iran J Pharm Res: IJPR. 2024;23(1):e152364.

19. Hoh C, Boocock D, Marczylo T, Singh R, Berry DP, Dennison AR, et al. Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. Clin Cancer Res. 2006;12(9):2944-50.

20. Koltai T, Fliegel L. Role of silymarin in cancer treatment: facts, hypotheses, and questions. J Evid Based Integr Med. 2022;27:2515690X211068826.

21. Koushki M, Yekta RF, Amiri-Dashatan N. Critical review of therapeutic potential of silymarin in cancer: A bioactive polyphenolic flavonoid. J Funct Foods. 2023;104:105502.

22. Exir Nano Sina. Sinaliv 70 [Internet]. Iran: Exir Nano Sina. Available from: https://ens.co.ir/products.

23. Guo Y, Xiong B-H, Zhang T, Cheng Y, Ma L. XELOX vs. FOLFOX in metastatic colorectal cancer: An updated meta-analysis. Cancer invest. 2016;34(2):94-104.

24. U.S. Department of Health and Human Services [Internet]. Common terminology criteria for adverse events (CTCAE) v 5.0; 2017 [cited 2025 Oct 05]. Available from: https://dctd.cancer.gov/research/ctep-trials/for-sites/adverse-events/ctcae-v5-5x7.pdf

25. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. Stat Methods Med Res. 2016;25(3):1057-73.

26. Abbasinia H, Heshmati M, Yousefi M, Najjar N, Sadeghi H. Silymarin-loaded tin(IV) nanoparticles exhibit enhanced bioavailability and antiproliferative effects on colorectal cancer cells. ACS Appl Bio Mater. 2023;6(9):3768-77.

27. Blayney DW, Schwartzberg L. Chemotherapy-induced neutropenia and emerging agents for prevention and treatment: a review. Cancer Treat Rev. 2022;109:102427.

28. Xiao AT, Tong YX, Xu XS, Zhou Y, Zhang S. Preoperative nutritional status contributes to the development of neutropenia event in patients with gastric cancer receiving CAPEOX adjuvant chemotherapy. Front Oncol. 2020;10:692.

29. Phua VCE, Wong WQ, Tan PL, Bustam AZ, Saad M, Alip A, et al. Capecitabine pattern of usage, rate of febrile neutropaenia and treatment related death in asian cancer patients in clinical practice. Asian Pac J Cancer Prev. 2015;16(4):1449-53.

30. Khan M, Alharbi S, Aljuhani S, Tunkar M, Morya A, Alnatsheh A, et al. The Incidence of Hematological Toxicities in Colorectal Cancer Patients Treated With Fluoropyrimidine-Based Regimens at Princess Noorah Oncology Center. Cureus. 2023;15(8):e44267.

31. Ghazanfar H, Nawaz I, Ali N. Oxaliplatin-Induced Thrombocytopenia: A Case Report and Review of Pathophysiology of Various Speculative Mechanisms. Cureus. 2020;12(8):e9929.

32. Lees JG, White D, Keating BA, Barkl-Luke ME, Makker PG, Goldstein D, et al. Oxaliplatin-induced haematological toxicity and splenomegaly in mice. Plos one. 2020;15(9):e0238164.

33. Gao A, Zhang L, Zhong D. Chemotherapy-induced thrombocytopenia: literature review. Discov Oncol. 2023;14(1):10.

34. Kato N, Nakai T, Kodama S, Koyama S, Nakane S, Wada Y, et al. Risk Factors for Thrombocytopenia Induced by Capecitabine Plus Oxaliplatin Therapy in Patients With Colorectal Cancer. in vivo. 2024;38(3):1243-52.

35. Curtis BR. Drug-induced immune thrombocytopenia: incidence, clinical features, laboratory testing, and pathogenic mechanisms. Immunohematology. 2014;30(2):55-65.

36. Abdel-Razeq H, Hashem H. Recent update in the pathogenesis and treatment of chemotherapy and cancer induced anemia. Crit Rev Oncol Hematol. 2020;145:102837.

37. Cobo F, De Celis G, Pereira A, Latorre X, Pujadas J, Albiol S. Oxaliplatin-induced immune hemolytic anemia: a case report and review of the literature. Anticancer drugs. 2007;18(8):973-6.

38. Sideris S, Loizidou A, Georgala A, Lebrun F, Gil T, Awada P, et al. Autoimmune haemolytic anaemia in a patient treated with capecitabine. Acta Clin Belg. 2013;68(2):135-7.

39. Yunan T, Yuke X, Guoran W, Dong W, Huifeng Z, Tao W, et al. Effects and mechanisms of Bazhen decoction, Siwu decoction, and Sijunzi decoction on 5-fluorouracil-induced anemia in mice. J Tradit Chin Med. 2016;36(4):486-95.

40. Salem S, Abd El-Baky A, Mohammed F. Cytoprotective effect of silymarin on cisplatin induced hepatotoxicity and bone marrow toxicity in rats. Asian J Anim Sci. 2017;11(3):140-152.

41. Ferraz AC, Almeida LT, da Silva Caetano CC, da Silva Menegatto MB, Lima RLS, de Senna JPN, et al. Hepatoprotective, antioxidant, anti-inflammatory, and antiviral activities of silymarin against mayaro virus infection. Antiviral Res. 2021;194:105168.

42. Baghbahadorani FK, Miraj S. The impact of Silymarin on improvement of platelet abnormalities in patients with severe preeclampsia. Electron Physician. 2016;8(5):2436-42.

**PLEASE CITE THIS PAPER AS:**

Karbasforooshan H, Rahimi H, Arasteh O, Allahyari A, Varmaghani M, Elyasi S. Efficacy of Oral Nano-Silymarin Formulation in the Prevention of Bone Marrow Suppression Induced by XELOX or m-FOLFOX6 Regimens in Metastatic Colorectal Cancer J Pharm Care 2025;13(2):150-161.

DOI: 10.18502/jpc.v13i3.20318