Running title: Nanocurcumin in bladder cancer patients receiving induction chemotherapy

# Randomized, Double-blind Pilot Study of Nanocurcumin in Bladder Cancer Patients Receiving Induction Chemotherapy

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## Abstract

**Purpose:** To evaluate the feasibility and potential efficacy of nanocurcumin supplementation in patients with localized muscle-invasive bladder cancer (MIBC) undergoing induction chemotherapy.

**Materials and methods:** In this double-blind, placebo-controlled trial, 26 MIBC patients were randomized to receive either nanocurcumin (180 mg/day) or placebo during the course of chemotherapy. All patients were followed up to four weeks after the end of treatment to assess the complete clinical response to the chemotherapy as primary endpoint. Secondary endpoints were the comparisons of chemotherapy-induced nephrotoxicity, hematologic nadirs, and toxicities between the two groups. Hematologic nadirs and toxicities were assessed during the treatment.

**Results***:* Nanocurcumin was well tolerated. The complete clinical response rates were 30.8 and 50% in the placebo and nanocurcumin groups, respectively. Although nanocurcumin was shown to be superior to placebo with respect to complete clinical response rates as the primary endpoint, there was no significant difference between the groups ($p = 0.417$). No significant difference was also found between the two groups with regard to grade 3/4 renal and hematologic toxicities as well as hematologic nadirs.

**Conclusion:** These preliminary data indicate the feasibility of nanocurcumin supplementation as a complementary therapy in MIBC patients and support further larger studies. Moreover, a substantial translational insight to fill the gap between the experiment and clinical practice in the field is provided.

## Introduction

Bladder cancer is one of the most common forms of cancer in men and women worldwide [1]. Despite several efforts to improve the treatment outcome of patients with muscle-invasive bladder cancer, the complete response rate of neoadjuvant chemotherapy remains to be around 30% over the last decade [2]. Therefore, an effective complementary therapy to the chemotherapy is needed to achieve better outcomes.

In some *in vitro* and *in vivo* studies, substantial chemosensitization by curcumin has been shown in the bladder tumor cells as well as many other cancers [3-15]. The emergence of such preclinical evidence for synergistic effect of curcumin with other chemotherapeutic drugs makes it a potential complementary therapy to be used in clinical practice [16]. At the cellular level, synergistic anticancer effect of SinaCurcumin® in combination with cisplatin has also been shown [15].

In spite of several promising roles for the curcumin in preclinical models, clinical evidence is equivocal due to inappropriate clinical pharmacokinetics such as low oral bioavailability and rapid metabolism [17]. Novel drug delivery systems have recently been designed to improve pharmacokinetics of anticancer phytochemicals including curcumin [18]. Curcumin nanoformulations have shown substantial potential to overcome the problem [19]. In a recent study, Yu et al. have reviewed the recent progress of the drug delivery systems aiming at bladder cancer therapy [20].

Recently, various clinical trials have focused on the use of SinaCurcumin®, a novel nanoformulation of curcuminoids, in different settings and disorders [21-24]. The results of these clinical studies are encouraging and suggest further research. Although some larger studies have investigated the complementary role of nanocurcumin in diverse range of cancer patients, but its value in the setting of bladder cancer patients undergoing induction chemotherapy has not yet been

assessed. Therefore, we conducted this pilot randomized trial to investigate the feasibility and clinical efficacy of nanocurcumin in this setting. In the light of this pilot study, further larger definitive trials could clarify the clinical utility of nanocurcumin as a complementary therapy in these patients.

## Material and Methods

*Study design*

This stratified, randomized, double-blind, placebo-controlled pilot study was conducted at two tertiary hospitals in Tehran, Iran, from September 2016 to February 2018. Consecutive male and female patients with histologically confirmed muscle-invasive bladder cancer (clinical stage T2-T4a) who had undergone initial transurethral resection of bladder tumor (TURBT) were assessed for eligibility. All patients were candidates for platinum-based neoadjuvant chemotherapy and have the performance status of 0 or 1. All participants had to have adequate baseline bone marrow and hepatic function. Patients with metastatic disease (N+, M+) were excluded from the study. The protocol of this study was in accordance with the Helsinki declaration and approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1396.227). All patients provided signed informed consent before inclusion in the study. This trial was registered on www.irct.ir (Identifier: IRCT20180226038875N1). Since Julious (2005) recommended a sample size of 12 per group as a rule of thumb in pilot studies, we planned to enroll a total of 24 participants in two groups [25]. Eligible patients were randomly assigned to one of two parallel groups in a 1:1 ratio to receive chemotherapy with either oral capsule nanocurcumin (SinaCurcumin®) or placebo using an internet-generated randomization list prepared by an investigator with no clinical involvement in the trial. The participants were stratified by the chemotherapy regimens (gemcitabine/cisplatin vs. gemcitabine/carboplatin) and

treatment centers. The nanocurcumin capsules were prepacked in sequentially numbered containers according to the randomization list. The randomization list was concealed from patients and all clinical investigators. An off-site person has labeled the drug packages with coded numbers. Each block of eight numbers was transmitted from the central office to an independent individual (a person not involved in the patient recruitment and treatment) in each center. Each participant was assigned an order number and received the capsules in the corresponding numbered containers.

*Treatment schedule*

All participants were randomized to receive chemotherapy with either oral capsule nanocurcumin 80 mg (SinaCurcumin®) or placebo two times daily. SinaCurcumin®, a commercially available capsule containing curcuminoids as nanomicelles, was prepared by the Exir Nano Sina Company, Tehran, Iran. Placebo capsules were provided by the same company as nanocurcumin and were perfectly matched in size, shape, odor, and color. The mean diameter of nanomicelles is around 10 nm, according to dynamic light scattering. Pharmacokinetic features of SinaCurcumin® have been recently published [(26)]. The authors declared that the bioavailability of SinaCurcumin® as a nanomicelle was estimated to be 59.2 times higher than its free form. All patients were administered gemcitabine (1000 mg/m$^2$, intravenous (IV), 30 min) on days 1 and 8, every 21 days. Following gemcitabine administration, patients with a creatinine clearance >60 ml/min received cisplatin on days 1 and 2 every 21 days (70 mg/m$^2$, 60 min) whereas those with a creatinine clearance <60 ml/min received carboplatin (AUC = 5, IV over 30 min) on day 1 every 21 days. Carboplatin doses were adjusted for renal function as per label using the Cockcroft-Gault formula.

*Outcomes and assessment*

The primary endpoint was complete clinical response to the chemotherapy as assessed four weeks after the end of treatment, using cystoscopy procedure performed by an independent blinded urologist. Eligible patients who received the treatment and had the follow-up tumor assessment were assessable for response. A clinical complete response to the induction chemotherapy defined as no evidence of primary tumor (T0) on cystoscopic assessment with biopsy. Complete blood count (CBC) test, hemoglobin, platelets, creatinine level, serum urea, liver enzymes, C-reactive protein, and erythrocyte sedimentation rate were evaluated at baseline, prior to each chemotherapy cycle, and after the treatment course.

Secondary endpoints were the comparisons of chemotherapy-induced nephrotoxicity, hematologic toxicities, and nadirs between the two groups. The lowest level of the hematologic parameters obtained after start of the chemotherapy course was selected as the nadir value. All toxicities were graded using the National Cancer Institute (NCI) common terminology criteria for adverse event (CTCAE) version 4.03.

*Statistical analyses*

Pearson's chi-square and Fisher's exact tests were used to compare the complete response rate as well as toxicities between the nanocurcumin and placebo groups. The baseline-adjusted hematologic nadirs were compared by analysis of covariance (ANCOVA) test. Mann-Whitney U test and Fisher's exact test were used for between-group comparison of the baseline characteristics. Missing data were handled by the complete case analysis. The statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, N.Y., USA). All statistical tests were performed at the two-tailed 5% level of significance.

## Results

*Patient characteristics*

From September 2016 to February 2018, a total of 36 patients were assessed for eligibility. Of these, 26 patients were included in the study and randomized to receive placebo or nanocurcumin (Figure 1). Patient demographics are summarized in Table 1. Nanocurcumin was well tolerated. One patient in the nanocurcumin group had a cardiac arrest from previously unrecognized 3-vessel coronary artery disease. Cisplatin-related pulmonary infection, renal failure, and skin rash were also reported in three patients receiving nanocurcumin.

*Tumor response*

Using a per-protocol approach, 13 patients in the placebo group and 10 patients in the nanocurcumin group were analyzed for treatment response. Tumor downstaging to pT0 was achieved in 39.1% of all patients. The complete clinical response rate was 30.8% (4/13) in the placebo group and 50% (5/10) in the nanocurcumin group. Although nanocurcumin was shown to be superior to placebo with respect to the primary endpoint, there was no significant difference between the groups ($p = 0.417$). There was no significant difference between the patients receiving cisplatin and carboplatin regarding the complete clinical response rate ($p = 0.999$).

*Hematologic nadirs and toxicity*

Secondary outcomes were analyzed on an intention-to treat basis and all patients receiving treatment ($n = 26$) were included in the toxicity assessment. The baseline-adjusted hematologic nadirs in the placebo and nanocurcumin groups are indicated in Table 2. No significant differences were found in the nadir levels between the two groups. Grade 3/4 toxicities are indicated in Table 3. As shown in the Table, no significant differences were observed between the two groups in terms of grade 3/4 renal and hematologic toxicities.

## Discussion

Chemotherapeutic agents e.g. cisplatin could induce inflammatory responses and this inflammation may reduce the treatment efficacy [27]. Hence, targeting of inflammation via combined use of anti-inflammatory agents and conventional cancer therapy is a rational approach [28,29]. Several studies have shown that curcumin could inhibit pro-inflammatory and inflammatory factors e.g. nuclear factor-kappa B (NF-κB), and consequently result in chemosensitization in the cancer cells as well as chemoprotection in the normal cells [12,30-33]. Furthermore, a series of target molecules e.g. apoptosis-related proteins, adhesion molecules, transcription factors, growth factors, and some key enzymes such as cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) may be involved in this dual function [30,31,34].

The direct antitumor activity of curcumin in bladder cancer cells has also been shown in several previous studies *in vitro* and *in vivo* [35,36]. The post-transcriptional activity of curcumin via down-regulation of miR-7641 and subsequent up-regulation of p16 has been reported in bladder cancer cells [37]. Wang et al. (2018) concluded that this regulation could lead to the decreased invasion and increased apoptosis of the bladder cancer cells. The administration of curcumin, as a chemopreventive agent, following the BCG therapy of bladder cancer has also been suggested by Hauser et al. (2007) [36].

Several promising preclinical studies have reported the complementary role of curcumin in combination with chemotherapy in different types of cancer [3-7]. Du et al. (2006) indicated a synergism between curcumin and 5-fluorouracil in HT-29 cell line, associated with a 6-fold reduction in the expression of COX-2 protein [3]. Dhandapani et al. (2007) showed that curcumin could suppress the cancer cell growth and chemoresistance of several chemotherapeutic agents such as cisplatin [4]. Also, the reduced chemoresistance of both cisplatin-resistant and wild-type cancer cells was also reported by Montopoli et al. (2009) [5]. In the mentioned study and another

*in vitro* study on MCF-7 and MDA-MB-231 cells [6], the cell cycle inhibition and apoptosis induction were observed. Furthermore, Zhang et al. (2018) demonstrated that curcumin in combination with cisplatin could significantly decrease proliferation and increase the apoptosis of A549 cells [8]. The authors also indicated that curcumin may hinder copper influx and increase uptake of platinum ion in cancer cells. They concluded that the process of chemosensitization to cisplatin therapy is regulated by the Cu-Sp1-CTR1 regulatory loop. In a former study, the direct antitumor activity of curcumin as a copper chelator is also described by Zhang et al. (2016) [38].

In preclinical models, the complementary role of curcumin in combination with antineoplastic agents has been well documented in bladder cancer cells [9-12]. In an *in vitro* study, Amanolahi et al. (2018) reported the synergistic effect of Curcumin and mitomycin in bladder cancer cells [9]. In their study, curcumin significantly decreased cell viability with increasing curcumin concentrations. They also declared that beside the antineoplastic activity in cancer cells, curcumin could protect normal cells from adverse effects of mitomycin. Although the potential chemoprotective role of curcumin against chemotherapy-induced myelosuppression and nephrotoxicity has been previously discussed in the literature [30,32,33], no protective effect was established in this study. Moreover, Afsharmoghadam et al. (2017) indicated a concentration-dependent effect of curcumin on antineoplastic activity of 5-fluorouracil in bladder cancer cells [10]. Their results suggest a critical role for curcumin concentration in the degree of cytotoxicity induced by chemotherapeutic agents. In another study, Park et al. (2016) explored the synergistic effect of curcumin combined with cisplatin to induce apoptosis in 253J-Bv (p53 wild-type) and T24 (p53 mutant) bladder cancer [11]. In both p53 wild-type and mutant bladder cancer cells treated with combination therapy, the apoptosis rate was increased compared to that in cells exposed to monotherapy. This synergistic interaction was found to be associated with the activation of

reactive oxygen species (ROS) and extracellular regulated kinase (ERK) signaling. The authors hypothesized that curcumin and cisplatin combination therapy can be an effective and reliable approach for the management of human bladder cancer. Beside the cisplatin, a significant synergistic inhibitory effect of curcumin in combination with gemcitabine and carboplatin, as two other agents used in this study, was also reported [12,13]. For instance, the combined apoptotic effect of curcumin and gemcitabine in bladder cancer cells was investigated by Kamat et al. (2007) [12]. They interestingly found that curcumin suppressed the gemcitabine-induced NF-κB activation in the bladder cancer cells.

While mentioned studies have addressed the complementary role of curcumin as a chemosensitizer in preclinical models, several gaps between the experiment and clinical practice remain to be filled. Recent studies have indicated that the poor clinical pharmacokinetics of curcumin could be improved using novel drug delivery systems e.g. nanoformulations [17-19]. In a recent study by Cheng et al. (2018) curcumin and cisplatin were co-encapsulated into the nanoliposomes [14]. The encapsulated curcumin and cisplatin as nanoparticles indicated the higher antineoplastic activity in comparison with free drug or encapsulated mono-drug therapy. In a recent *in vitro* study on SinaCurcumin®, synergistic antineoplastic effect of high dose nanocurcumin in combination with cisplatin has been reported [15]. Importantly, the authors declared that the effect was dose- and cell type-dependent. In the light of this pilot trial, further clinical study is needed to determine the suitable effective doses of nanocurcumin in the clinical setting.

The lack of enough power and statistical significance to accept or reject the study hypothesis may expectedly be associated with the small sample size as the main limitation of the study. The authors acknowledge that the small sample size of the trial prevents any meaningful inferences based on the study results. However, the present study describes the first clinical experience of

nanocurcumin in bladder cancer patients undergoing chemotherapy and provides new insight into future clinical directions. Additional trials with a large number of patients will explain whether the nanocurcumin acts as a complementary therapy in these patients. Considering several experiences with different times of SinaCurcumin® supplementation [21-24], we believe that a longer duration of supplementation seems to yield a better outcome. In a future well-designed trial, the pathologic response to chemotherapy could be used as a stronger surrogate endpoint. Since the majority of patients with the complete clinical response in the study received chemo-radiation instead of surgery procedure, the assessment of pathologic response as the primary endpoint was impossible in our study. In order to high rate of false positive results, CT scan and MRI are rarely used in the clinical setting after neoadjuvant chemotherapy [39]. Moreover, considering the role of distinct Genetic subtypes of bladder cancer in sensitivity of tumor to frontline Chemotherapy, the identification of different subtypes of bladder tumors may be helpful in future studies [40,41]. Finally, as the first report in the setting, this report summarized the possible mechanisms of action and some clinical directions for future investigations.

**Conclusions**

These preliminary data suggest feasibility of nanocurcumin supplementation in this clinical setting and support further larger studies. This pilot study may also provide a substantial translational insight to fill the gap between the experiment and clinical practice. A large-scale randomized trial in the setting is warranted.

**Conflict of Interest**

The authors have declared that there is no conflict of interest.

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Figure Legend:

Figure 1. The CONSORT flow chart of patients

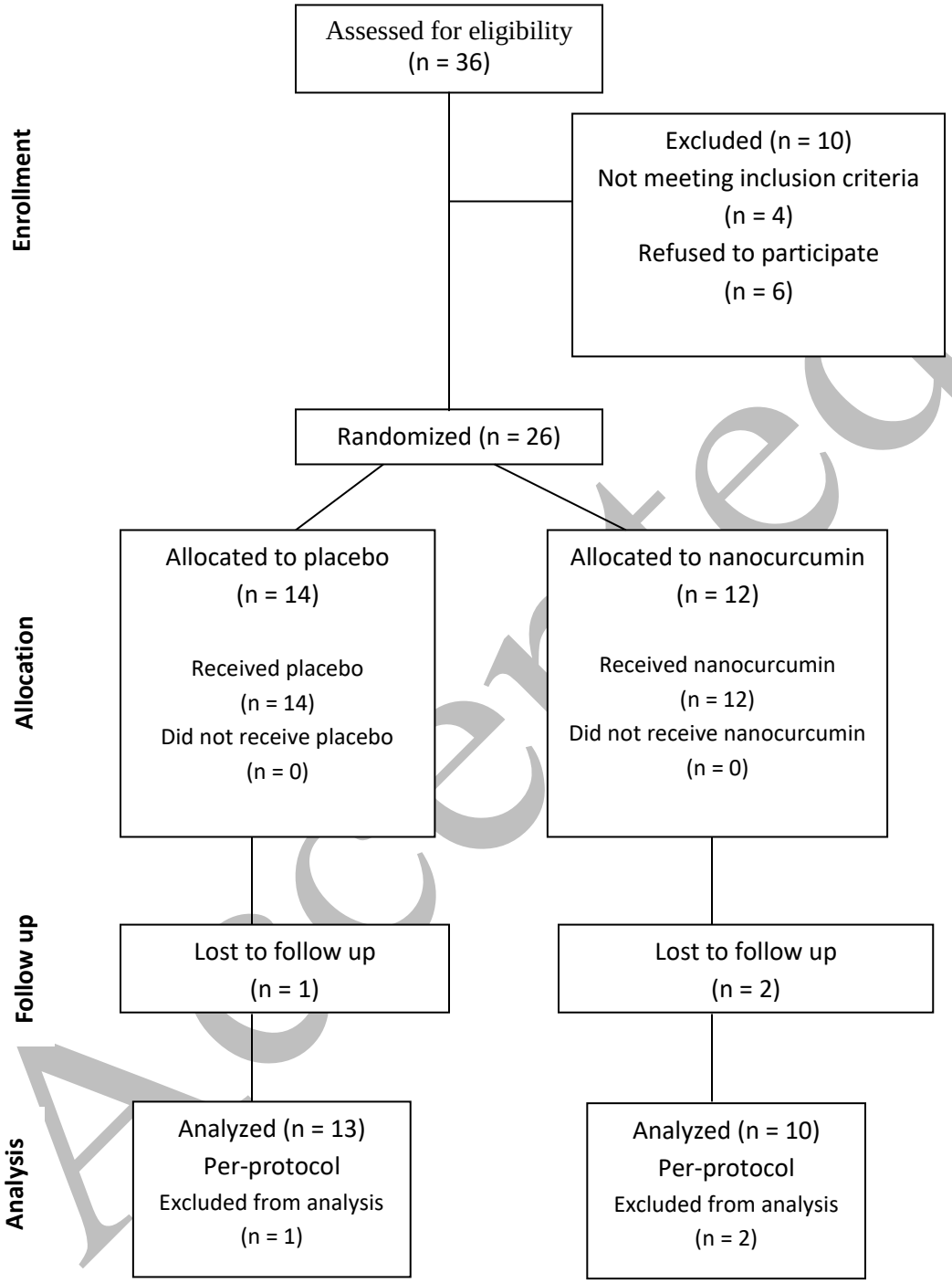


Figure 1. The CONSORT flow chart of patients

Table 1. Baseline characteristics of patients

| **Characteristics** | **Placebo (*n* = 14)** | **Nanocurcumin (*n* = 12)** | ***P*-value** |
|---|---|---|---|
| Age, mean (SE), years | 64.7 (2.4) | 68.2 (3.7) | 0.462[a] |
| Height, mean (SE),cm | 166.5 (2.9) | 169.5 (1.9) | 0.595[a] |
| Weight, mean (SE), kg | 77.0 (4.9) | 74.3 (2.7) | 0.999[a] |
| Regimen, n (%) | | | |
| Gem/Cis | 8 (57.1) | 8 (66.7) | 0.701[b] |
| Gem/Carbo | 6 (42.9) | 4 (33.3) | |
| Creatinine clearance, mean (SE), mL/min | 59.3 (7.3) | 62.6 (5.8) | 0.432[a] |
| Hemoglobin, mean (SE), g/dl | 13.0 (0.5) | 13.5 (0.4) | 0.899[a] |
| Platelets, mean (SE), $10^9$/L | 277 (20) | 240 (15) | 0.131[a] |
| Leucocytes, mean (SE), $10^9$/L | 7.9 (0.7) | 8.2 (0.6) | 0.467[a] |

SE, Standard Error of mean; Gem, Gemcitabine; Cis, Cisplatin; Carbo, Carboplatin

[a] Mann-Whitney U test was used

[b] Fisher's exact test was used

Table 2. Hematologic nadirs with adjustment for baseline values

| **parameter** | **Nadir value [a], mean (SE)** | | **Between-group difference, mean (95% CI)** | ***P*-value [b]** |
|---|---|---|---|---|
| | **Placebo** | **Nanocurcumin** | | |
| Leucocytes | 3.3 (0.1) | 3.0 (0.2) | 0.3 (-0.2 to 0.9) | 0.203 |
| Neutrophils | 1.2 (0.2) | 1.0 (0.2) | 0.2 (-0.2 to 0. 8) | 0.323 |
| Lymphocytes | 1.7 (0.2) | 1.3 (0.2) | 0.4 (-0.2 to 0.9) | 0.177 |
| Hemoglobin | 9.5 (0.4) | 10.2 (0.4) | 0.7 (-0.7 to 0.1.9) | 0.354 |
| Platelets | 170 (7) | 167 (7) | 3 (-18 to 26) | 0.732 |
| Creatinine clearance | 56.4 (3.8) | 47.7 (4.1) | 8.7 (-3.1 to 20.5) | 0.142 |

[a] Units of the parameters: creatinine clearance (mL/min); leucocytes, neutrophils, lymphocytes, and platelets ($10^9$/L); hemoglobin level (g/dL)
[b] ANCOVA test was used for all parameters with adjustment for baseline values of each parameter

Table 3. Chemotherapy-induced toxicities (Grade 3/4)

| Endpoint | Placebo group (n = 14) | Nanocurcumin group (n = 12) | *P*-value |
|---|---|---|---|
| Leukopenia, n (%) | 2 (14.3) | 1 (8.3) | 0.999[a] |
| Neutropenia, n (%) | 5 (35.7) | 7 (58.4) | 0.249[b] |
| Anemia, n (%) | 1 (7.1) | 1 (8.3) | 0.999[a] |
| Thrombocytopenia, n (%) | 0 | 0 | - |
| Nephrotoxicity, n (%) | 1 (7.1) | 2 (16.7) | 0.580[a] |

[a] Fisher's exact tests was used
[b] Pearson's chi-square test was used