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Effectiveness of crocin of saffron (*Crocus sativus* L.) against chemotherapy-induced peripheral neuropathy: A randomized, double-blind, placebo-controlled clinical trial

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ABSTRACT

Ethnopharmacological relevance: Chemotherapy-induced peripheral neuropathy (CIPN) is one of the complications vexes patients treated with anti-cancer agents. Saffron has been demonstrated to attenuate symptoms of peripheral neuropathy in animal models. Also, there is a published clinical trial that investigated the pain relieving effect of saffron following nationally accepted rules and concluded that saffron was successful in alleviating pain symptoms in patients suffering from fibromyalgia.

Aim of the study: We aimed to determine the efficacy of crocin as a constituent of saffron in CIPN as the first report.

Materials and methods: One hundred and seventy-seven enrolled eligible patients (between December 2018 and March 2020) for study entry were cases demonstrating mild to severe symptomatic CIPN for at least a month. These cases were randomly assigned to two main groups including 15 mg crocin tablet, bid (30 mg total daily target dose) and placebo tablet for 8 weeks. A crossover study was performed with a 2-week washout period. Patient outcomes were measured once a week for 8 consecutive weeks.

Results: Grade of sensory, motor and neuropathic pain decreased considerably and significantly in the crocin group compared with placebo ($P < 0.05$). Observed toxicities were mild and adverse effects had no significant differences between the two groups ($P > 0.05$).

Conclusions: Crocin considerably seems to be effective for relieving symptoms of CIPN in cancer patients receiving chemotherapy agents. However, further studies are needed about crocin with its beneficial neuropharmacological effects and lower adverse effects than the chemical agents such as antidepressants, lamotrigine, and gabapentin.

1. Introduction

1.1. Background

Cancer is one of the leading causes of death and disability in the world and leads to approximately 7.6 million deaths per year (Gonzalez and Riboli, 2010). One of the main treatment options for cancer is chemotherapy, which is still widely used (Liao et al., 2013).

Neuropathic pain is a neurological problem that affects the structure and function of the sensory system and causes abnormal pain and pathological activity in various parts of the body (Safakhah et al., 2016). Damage to the sensory nerves includes loss of sensation, paresthesia, dysesthesia, tingling, and in severe cases appear as neuropathic pain (Kerckhove et al., 2017). Neuropathic pain is mainly associated with the following 2 abnormal sensory parameters; 1) Hyperalgesia (sensitivity to painful stimuli). 2) Allodynia (pain caused by a stimulus that does not

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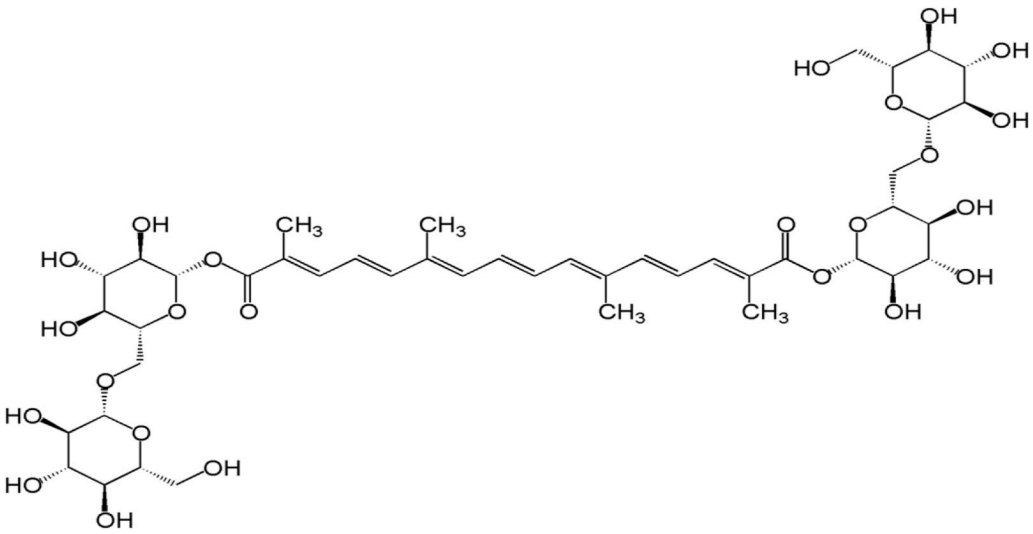

Fig. 1. Chemical structure of crocin.

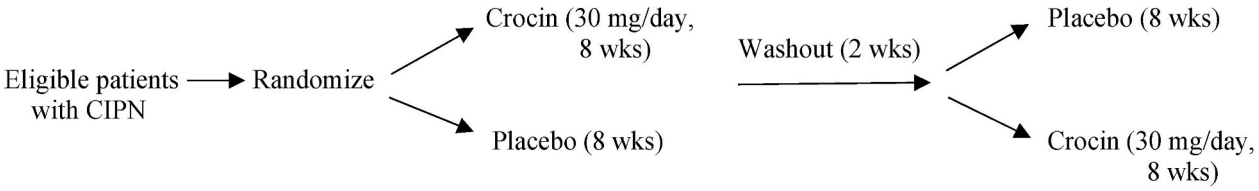

Fig. 2. Illustration demonstrated study design.

normally occur in healthy people) (Safakhah et al., 2016).

One of the causes of neuropathic pain is chemotherapy that termed it chemotherapy-induced peripheral neuropathy (CIPN). This common and debilitating complication is caused by the use of compounds such as vinca alkaloids, taxan derivatives and platinum compounds, the extent of which varies depending on the dose per cycle, the cumulative dose, and the duration of chemotherapy injections (Kerckhove et al., 2017; Schloss et al., 2017; Ewertz et al., 2015). About 38% of patients who undergo chemotherapy develop this complication in the long term, which increases to 90% if oxaliplatin is used (Kerckhove et al., 2017). Neuropathy also occurs in 30% of patients treated with taxanes, and may persist for several years after chemotherapy. CIPN is irreversible due to the use of cisplatin (Ewertz et al., 2015). The most common symptoms of CIPN that may continue or even increase for a long time after stopping chemotherapy include pain, tingling, numbness, difficulty concentrating, and short-term memory loss. However, symptoms such as impaired mobility, autonomic dysfunction, and skull nerve involvement may occur in some cases (Kerckhove et al., 2017; Ewertz et al., 2015; Miltenburg and Boogerd, 2014).

Many common strategies for preventing and treating CIPN are not very effective today. Oncologists are forced to reduce the dose of chemotherapy drugs and sometimes cease treatment to improve neurological symptoms, which can affect patient survival (Kerckhove et al., 2017; Ewertz et al., 2015; Miltenburg and Boogerd, 2014). Treatment options for CIPN include adjuvant therapy such as tricyclic antidepressants (nortriptyline, amitriptyline, and imipramine), anticonvulsants (gabapentin and pregabalin), topical agents such as a composite topical gel containing baclofen, amitriptyline, opioids, NSAIDs, and ketamine that have shown limited effects (Ewertz et al., 2015; Miltenburg and Boogerd, 2014; Piccolo and Kolesar, 2014). Glutathione and vitamin E have also been used to prevent CIPN but have not had a significant positive effect. Calcium and magnesium injections have also been effective and are not commonly used due to reduced chemotherapy efficacy effects (Ewertz et al., 2015; Miltenburg and Boogerd, 2014; Piccolo and Kolesar, 2014). Recent studies show that duloxetine and to a lesser extent venlafaxine have positive effects in improving CIPN (Farschchian et al., 2018). It should be noted that medicinal plants should be considered for better availability, lower interaction with the chemical drugs, fewer adverse effects, and high relative effectiveness (Schloss et al., 2017; Piccolo and Kolesar, 2014).

Saffron (Crocus sativus L., called the king of the spices) is a Traditional Persian Medicine (TPM) and growing plant which is found in Khorasan province, Mashhad, Iran. Saffron has been enriched in minerals and trace elements (Rameshrad et al., 2018). Saffron is a plant from the lily family with many pharmacological effects and is considered as an old medicine. Safranal, crocin, crocetin, beta-carotene, lycopene, zaxatin, vitamins including riboflavin and thiamine are substances in saffron leaves, flowers, and stigmas. Over the past decade, findings show that saffron active ingredient has analgesic, antioxidant, anti-genotoxic, anti-tumor, anti-inflammatory, anticonvulsant, anti-depressant, anti--bacterial, sedative, memory-enhancing, and finally neuroprotective effects. This agent provides tissue oxygenation and strengthens the immune system (Safakhah et al., 2016; Rameshrad et al., 2018). Also, aqueous and ethanolic extracts of saffron and safranal can be used in the treatment of neuropathic pain in animal models as an adjuvant may be appropriate (Amin and Hosseinzadeh, 2012).

1.2. Objectives

There is a clear published scientific observation obtained on a clinical trial. This study investigated the pain relieving effect of saffron following nationally accepted rules and concluded that saffron was successful in alleviating pain symptoms in patients suffering from fibromyalgia (Shakiba et al., 2018). Therefore, considering the positive effects of saffron in the prevention or treatment of pain in human and animal models, we aimed to investigate the effects of crocin (with its chemical structure demonstrated in Fig. 1) as a constituent of saffron on CIPN. To the best of the author's knowledge, no clinical trial, as well as

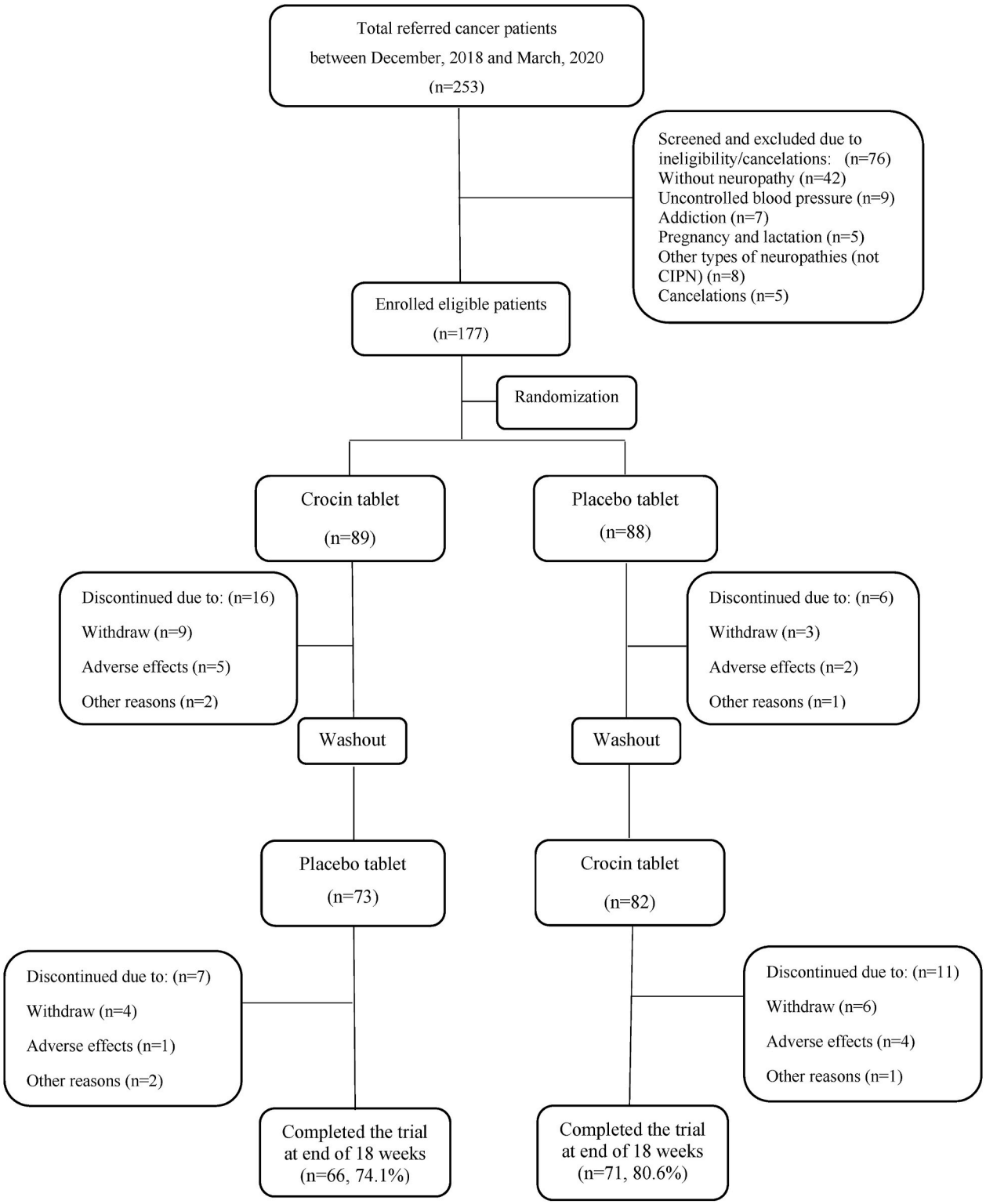

Fig. 3. Illustration demonstrating patient inclusion/exclusion and group distribution of 253 cancer patients during a crossover study with 2-week washout period.

laboratory animal study, hase been performed so far to evaluate the effects crocin on CIPN.

2. Materials and methods

2.1. Trial design

The present double-blind, placebo-controlled clinical trial was approved by Research Ethics Committee of Semnan University of Medical Sciences and Health Services (approval ID: IR.SEMUMS.REC.1397.195) and also registered with the Iranian Registry of Clinical Trials [(IRCT), registration number: IRCT20180119038433N1]. The present study was a randomized, double-blind, placebo-controlled, and crossover design consisting of two 8-week phases separated by a 2-week "washout'' period.

Table 1
Demographics and baseline patient characteristics with CIPN. Digits in the parentheses were expressed as percentage or range. C/P; the group that received crocin in the first 8-week period and placebo in the second 8-week period. P/C; the group that received therapy in the reverse order.

	C/P	P/C	Total	*P* value
	(n = 89)	(n = 88)	(n = 177)	
Age, y (mean ± S.E.M.)	61 ± 3.2 (27–84)	62 ± 3.8 (25–89)	61.5 ± 3.6 (25–89)	0.4
Sex				0.9
Female	48 (53.9)	46 (52.2)	94 (53.1)	
Male	41 (46)	42 (47.7)	83 (46.8)	
Malignancy type				0.6
Melanoma	2 (2.2)	3 (3.4)	5 (2.8)	
Prostate cancer	12 (13.4)	14 (15.9)	26 (14.6)	
Lymphoma	2 (2.2)	1 (1.1)	3 (1.6)	
Thyroid	9 (10.1)	7 (7.9)	16 (9)	
Breast	23 (25.8)	20 (22.7)	43 (24.2)	
Colorectal	14 (15.7)	11 (12.5)	25 (14.1)	
Lung	9 (10.1)	13 (14.7)	22 (12.4)	
Cervix	10 (11.2)	9 (10.2)	19 (10.7)	
Ovaries	5 (5.6)	7 (7.9)	12 (6.7)	
Leukaemia	3 (3.3)	3 (3.4)	6 (3.3)	
Type of chemotherapy				0.6
Active	42 (47.1)	46 (52.2)	88 (49.7)	
Discontinued or completed	47 (52.8)	42 (47.7)	89 (50.2)	
Chemotherapy regiment				0.5
Taxanes	32 (35.9)	29 (32.9)	61 (34.4)	
Platinum-based compounds	30 (33.7)	33 (37.5)	63 (35.5)	
Vincristine	9 (10.1)	7 (7.9)	16 (9)	
FOLFOX	10 (11.2)	11 (12.5)	21 (11.8)	
TPF	8 (8.9)	8 (9)	16 (9)	

2.2. Participants

Two hundred and fifty-three adult cancer patients who were referred to the Cancer Research Center of Kosar Hospital (Semnan) and 2 other centers [Oncology Clinic of Milad Hospital (Tehran), and Oncology Clinic of Shariatee Hospital (Tehran)], were examined from December 2018 to March 2020. These patients immediately received chemotherapy drugs and were examined for symptomatic neuropathy. Diagnosis of neuropathy based on physician's history, questionnaires, physical examination, electromyography (EMG), and nerve conduction velocity (NCV) tests. Of these patients, all 177 cases with symptomatic CIPN for at least a month duration with average daily symptom scores of 1) ≥4 on a 0–10 numerical rating scale (NRS; 0 = no pain and 10 = worst pain); or 2) ≥1 on the 0–4 World Health Organization (WHO) score (0 = none; 1 = paresthesia/decreased tendon reflexes; 2 = severe paresthesia/mild weakness; 3 = intolerable paresthesia/marked motor loss; 4 = paralysis); or 3) ≥1 on the 0–4 National Cancer Institute of Canada Common Toxicity Criteria (NCIC-CTC) score (0 = normal; 1 = subjective weakness/paresthesia; 2 = objective weakness/objective sensory loss or paresthesia; 3 = weakness/sensory loss/paresthesia interfering with activities; 4 = paralysis/permanent sensory loss that interferes with function); or 4) ≥1 on the 0–4 Eastern Cooperative Oncology Group (ECOG) Neuropathy Scale (ENS; 0 = none; 1 = decreased deep tendon reflexes/mild paresthesia; 2 = absence deep tendon reflexes/severe paresthesia; 3 = disable sensory loss/severe peripheral neuropathic pain; 4 = respiratory dysfunction due to weakness/paralysis) were eligible for study entry. According to a previous study, cancer patients almost show CIPN in the first month after chemotherapy with a prevalence of 68.1% (Seretny et al., 2014). In all cases symptomatic CIPN had been resulted from receiving one of the following neurotoxic chemotherapy agents; taxanes (paclitaxel, docetaxel), platinum-based compounds (oxaliplatin, cisplatin), vinca alkaloid (vincristine), and newer combinations (TPF; docetaxel/cisplatin/fluorouracil, and FOLFOX; oxaliplatin/fluorouracil/folinic acid). All eligible individual participants had filled the informed consent form before beginning the study.

Exclusion criteria were included cancelations, uncontrolled blood pressure, addiction, pregnancy, lactation, neuropathies due to other causes, except CIPN, like radiotherapy, plexopathy, disk protrusion, spinal stenosis, radiculopathy, vitamin B12 deficiency, and diabetes. In order to minimize errors, patients were contraindicated to use antidepressants, opioids, adjunct systemic or topical analgesic agents such as anticonvulsants, benzodiazepines, lidocaine, mexiletine, and amifostine. Therapy with the mentioned agents could be performed after study entry if necessary. Demographic and baseline characteristics of patients were collected including age, sex, malignancy type, type of chemotherapy (active, discontinued or completed), and type of neurotoxic chemotherapy regiment received.

2.3. Study settings

Stigmas of saffron were purchased from Talakaran-e-Mazraeh Co., (Torbate-Heydarieh, Razavi Khorasan province, Iran) and analyzed in accordance with the ISO/TS 3632–2. An aqueous saffron extract was prepared using the modified method described by Hadizadeh et al. (2010). The extract was standardized by crocin using the method defined by Hosseinzadeh et al. (2008) and crocin content of saffron extract was measured 2.94 (~3) mg/15 mg of extract (Because each crocin tablet was prepared with 15 mg of crocin). Furthermore, the placebo tablets were completely similar in shape, color, and size to the crocin tablets. The measurement of crocin in aqueous extract of saffron was carried out according to the modified method which was described previously (Hosseinzadeh and Noraei, 2009). Generally, the saffron extract was filtered through a 0.2 μm Millipore filter (Millipore, Bedford, MA, USA) and eluted with 100% methanol. This quantification was performed by Shimadzu HPLC LC-10ADvp system integrated with a Shimadzu SCL-10Avp system controller and a SPD-10Avp ultraviolet–visible spectrophotometric on a reversed-phase Shim-pack C18, VP-ODS analytical column (25 cm × 4.6 mm I.D. with a 12.0 ± 1.0 nm pore size and 4.6 ± 0.3 μm particle size), using an isocratic mobile phase of acetonitrile: water (76:24%) at a flow rate of 1.2 ml/min. A Rheodyne Shimadzu Model 7725i injector was used to inject 25 μl of the sample from a 25 μl Hamilton straight-edge needle syringe onto the column. All data were recorded and analyzed on a chromatography workstation using Shimadzu Class-VP™ 6.10 software. Finally, pure crocin powder was extracted by Mashhad University of Medical Sciences. The tablets, containing 20% crocin and 80% starch excipient, were manufactured and then an industrial pharmacist performed the quality tests on them.

Data were collected from 3 medical centers including Cancer Research Center of Kosar Hospital (Semnan), Oncology Clinic of Milad Hospital (Tehran) and Oncology Clinic of Shariatee Hospital (Tehran). Before the randomization of participants, they were classified by the kind of chemotherapeutic agents used (as mentioned before) and by whether they were enrolled during ongoing chemotherapy or had completed their therapy.

2.4. Interventions

Participants were randomly treated with 15 mg crocin tablet, twice daily (target dose of 30 mg/day) versus an identical-appearing placebo tablet at bedtime for 8 weeks. After 8 weeks of therapy from the time of drug initiation, patients were stopped to use of crocin/placebo over a 2-week period and then the drug administration order between crocin and placebo had been reversed. Since, there is no clear study indicate the exact dose of crocin in CIPN, we used the nearest published data that suggests the mentioned effective and safe dose (Mousavi et al., 2015). Crocin generally has no serious adverse effects but its consumption without discretion (overdose and/or long-term use) can lead to nausea and vomiting. Patients were monitored prospectively by weekly phone calls and questionnaires during the 8-week study period and the

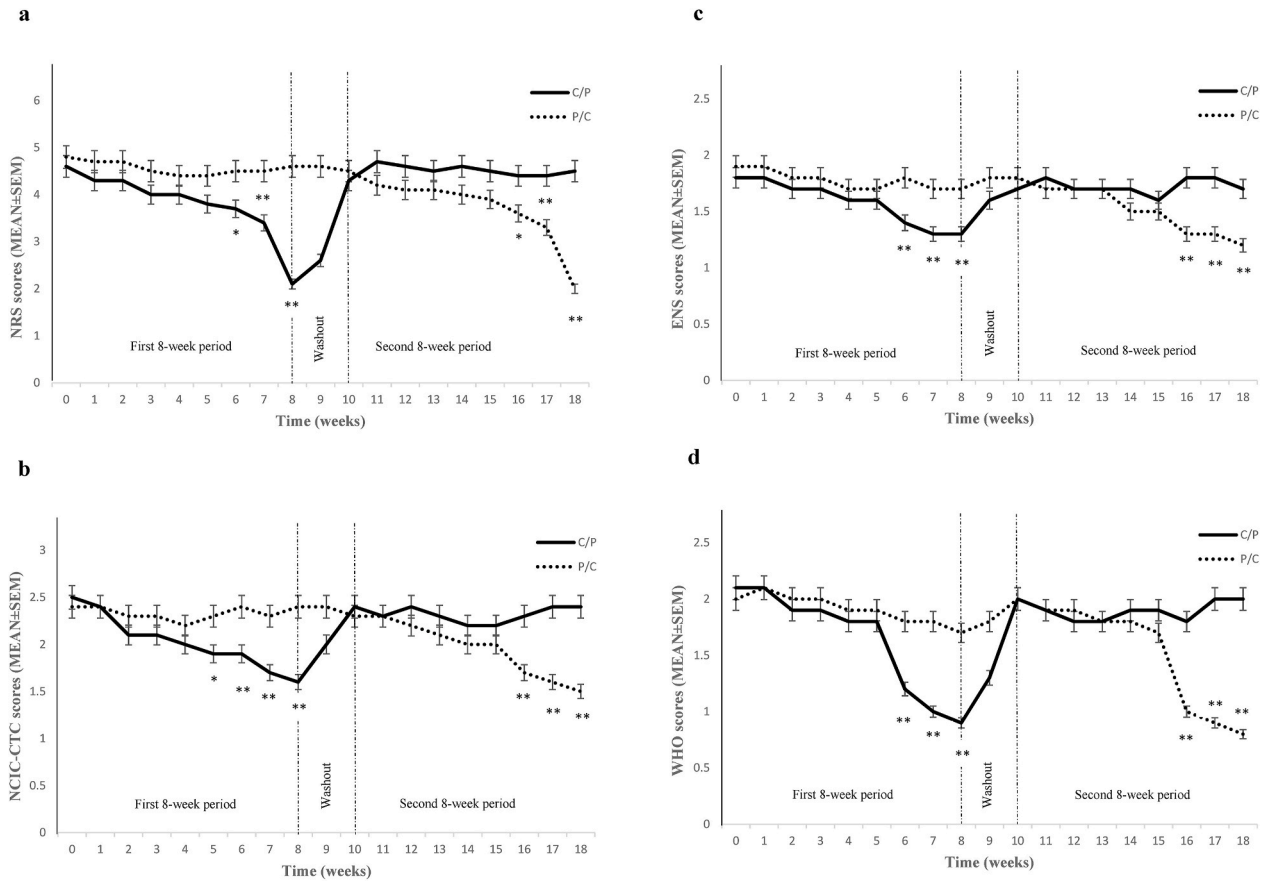

Fig. 4. NRS average pain score (a), NCIC-CTC grading scale (b), ENS average pain score (c), and WHO neuropathy scale (d) measured during the 18-week study period. As shown at the endpoints of some of the weeks, independent *t*-test showed significant differences between the two groups (*P < 0.05, **P < 0.01). C/P; the group that received crocin in the first 8-week period and placebo in the second 8-week period. P/C; the group that received therapy in the reverse order. Data were expressed as mean ± S.E.M.

outcomes were assessed.

2.5. Outcomes

The primary outcomes including the severity of neuropathic complaints [as for NRS (Rosenstock et al., 2004; Farrar et al., 2001), WHO (Miller et al., 1981), NCIC-CTC (Postma et al., 1998), and ENS (Oken et al., 1982) scores] as well as additional secondary efficacy measures were assessed. These secondary efficacy measures included; the Brief Pain Inventory (BPI) Short-Form; rapidly assesses the severity of pain and its influence on functioning (Cleeland and Ryan, 1994), McGill Pain Questionnaire (MPQ; assesses both quality and intensity of subjective pain) (Melzack, 1987), the Symptom Distress Scale (SDS; a 5-point scale developed to evaluate the construct of symptom distress, defined as the degree of discomfort from the specific symptom being experienced as reported by the patient) (McCorkle and Young, 1978), the Subjective Global Impression of Change (SGIC; evaluates health status as perceived by the patient in a 7-point scale ranging from 'very much worse' to 'very much improved') (Guy, 1976), the Quality of Life Scale (QOLS; s singleitem measurement of global QOL on a numerical analog scale from 0 to 100) (Bretscher et al., 1999), and Neuropathy Pain Scale (NPS; a 10-point scale that measures pain intensity. 0 = no pain, and 10 = the most pain imaginable) (Jensen et al., 2006). During this 8-week period, the investigator was advising the patient to the correct use of medications and also examined the possible adverse effects, and occurrence of drug reactions. There was no patient with intolerable complications that needed to discontinue the drug use during the study.

2.6. Sample size

We calculated the sample size using G*Power 3.1.9.2 (Heinrich-Heine-Universität, Dusseldorf, Germany), α error probability = 0.05, Power (1-β error probability) = 0.8, effect size = 0.2. We calculated that a minimum of 150 patients would be needed to show a difference in pain scores. However, we added 27 patients to our samples to have a higher confidence in our results. Also, since there is no similar clinical trial to ours, before beginning the study, we performed a pilot clinical study to calculate the effect size.

2.7. Randomization and blinding

Crocin and placebo tablets were prepared in a similar shape, color, and size and then kept in an enclosed container. All the tablets were coded by a pharmacist who is out of the study. Both treating researchers and patients were not knowing the code labeled on the container. The randomization was performed using a computer-generated random allocation list and the envelope method.

2.8. Statistical analysis

The collected data was recorded and analyzed for statistical analysis using Graphpad Prism (Graphpad Software Inc., San Diego, CA, USA).

Table 2
Secondary outcome analysis results for the crossover study in both groups. Digits in the parentheses were expressed as percentage. C/P; the group that received crocin in the first 8-week period and placebo in the second 8-week period. P/C; the group that received therapy in the reverse order. *P* values relate to comparisons between the 2 groups at the corresponding time point.* Higher scores indicate greater severity of symptoms.ẍ Higher scores indicate fewer symptoms or improvement. NRS; numerical rating scale. NCIC-CTC; National Cancer Institute of Canada Common Toxicity Criteria. ENS; Eastern Cooperative Oncology Group Neuropathy Scale. WHO; World Health Organization. BPI; Brief Pain Inventory. SDS; Symptom Distress Scale. SGIC; Subjective Global Impression of Change. QOL; Quality of life. NPS; Neuropathy Pain Scale.

	Baseline	P	End of 8 weeks	P	End of 18 weeks	P
Number per group		–		–		–
C/P	89		73		66	
P/C	88		82		71	
NRS average pain*		0.5		0.002		0.002
C/P	4.3		2.1		4.5	
P/C	4.7		4.6		2	
NCIC-CTC scale*		0.7		0.005		0.005
C/P	2.5		1.6		2.4	
P/C	2.4		2.4		1.5	
ENS average pain*		0.7		0.007		0.007
C/P	1.8		1.3		1.7	
P/C	1.9		1.7		1.2	
Mean WHO neuropathy score*		0.7		0.003		0.002
C/P	2.1		0.9		2	
P/C	2		1.7		0.8	
BPI average score*		0.6		0.009		0.005
C/P	3.4		2.7		3.1	
P/C	3.2		3.1		2.2	
McGill pain rating index*		0.8		0.005		0.003
C/P	25.3		14.6		23.9	
P/C	24.1		22.9		13.8	
Mean total SDS score*	0.8		0.009		0.008	
C/P	69.2		59.4		67.7	
P/C	67.4		66.6		56.9	
SGIC scaleẍ		–		0.000		0.000
C/P	–		0.9		0.3	
P/C	–		0.3		1.1	
QOL scaleẍ		0.1		0.009		0.008
C/P	61.3		75.5		63.2	
P/C	66.2		67.4		77.3	
Mean NPS*		0.7		0.005		0.002
C/P	3.2		2.1		3	
P/C	3.1		3		1.9	

3. Results

3.1. Participant flow

After screening of 253 cancer cases between December 2018 and March 2020, finally, 177 eligible patients were registered and randomized to the 2-arm crossover clinical trial. The overall scheme of the study and the Consort (Consolidated Standard for Reporting Clinical Trials) diagram are depicted in Fig. 2 and Fig. 3, respectively. A total of 89 and 88 patients were enrolled into the C/P arm and the P/C arm, respectively. Of these, 66 and 71 patients had completed the study, respectively.

Table 3
Frequency and severity of adverse effects attributed to therapy in both groups.

Adverse effects	Crocin	Placebo	*P*
Nausea			
Grade 2	3	1	0.2
Vomiting			
Grade 1	1	1	0.7
Stomach ache			
Grade 1	2	1	0.5
Swelling of feet			
Grade 1	1	0	0.4
Sedation			
Grade 1	3	2	0.5
Increased appetite			
Grade 3	5	4	0.5
Headache			
Grade 1	3	2	0.5
Hypomania			
Grade 1	2	2	0.7

3.2. Losses and exclusions

The main reasons for removing the enrolled patients were listed in Fig. 3. The patients had discontinued the protocol because of withdraw, adverse effects or other reasons.

3.3. Baseline data

Participants in both C/P and P/C arms were generally balanced as for demographic characteristics, cancer type, and type of chemotherapy regiment and drugs responsible for CIPN at baseline (Table 1). In this table, none of the mentioned items of patients, based on the stratification factors had a statistically significant difference between the C/P and P/C arms.

3.4. Numbers analyzed

66 and 71 patients in the C/P and P/C arms had completed the study, respectively and their data were recorded and analyzed.

3.5. Outcomes and estimation

As shown in Fig. 4(a–d), CIPN scores improved gradually for all patients receiving crocin during the trial, compared with their scores at baselines. These gradual improvements in CIPN scores also were seen in secondary scales during the trial (data were not shown). Paired *t*-test results showed a prominent and significant decrease in values at the end of 8th week compared to the baseline scores in C/P arms ($P < 0.01$). Reciprocally, mean scores at the end of 18th week were significantly lower than the baselines at the end of 10th week (beginning the second section of the study) in P/C arms ($P < 0.01$).

As shown in Table 2, at the beginning the study, the baseline scores for average pain using the NRS were 4.3 and 4.7 ($P = 0.53$) in the C/P and P/C arms, respectively. These baseline values were 2.5 and 2.4 ($P = 0.72$) by NCIC-CTC, 1.8 and 1.9 ($P = 0.72$) by ENS, and 2.1 and 2 ($P = 0.72$) by WHO scales in the C/P and P/C arms, respectively. Generally, there were no significant differences in baseline symptoms severity between the 2 arms using the above primary scales and the other secondary scales. At the end of the 8 weeks of therapy, symptom severity significantly decreased in C/P arms compared to P/C arms in all the related scales. Amount of these changes in mean values in C/P arms compared to P/C arms were −2.5 (54.3%) by NRS average pain, −0.8 (33.3%) by NCIC-CTC scale, −0.04 (23.5%) by ENS scale, −0.8 (47%) by WHO scale, −0.4 (12.9%) by BPI scale, −8.3 (36.2%) by McGill pain rating index, −7.2 (10.8%) by SDS, and −0.9 (30%) by NPS. Furthermore, scales that indicate the patient's improvement as the score increases, like SGIC and QOL also were measured. In these scales, the

amount of changes in mean values in C/P arms compared to P/C arms were +0.6 (66.6%) and +8.1 (10.7%) by SGIC and QOL scales, respectively. Conversely, at the end of the 18 weeks of therapy, P/C arms showed a significant decrease in CIPN severity ($P < 0.01$) and an increase in patient's recovery/improvement ($P < 0.01$) compared to C/P arms in all related scales. As shown in Fig. 4(a–d), the last 3 endpoints data for the C/P arms in the first 8-week period and the last 3 endpoints data for the P/C arms in the second 8-week period were significantly changed compared to the opposite arms.

3.6. Adverse effects/toxicities

Adverse effects occurred at relatively equivalent rates in both arms (Table 3). Adverse effects occurred at relatively equivalent rates in both groups of patients receiving crocin or placebo. There were no differences in the incidence of adverse effects in patients who were being administered with crocin and placebo and also there were no differences in the incidence of these annoying effects between the C/P and P/C arms. Refusals and adverse events were slightly higher in patients receiving crocin than were patients on placebo (15.7% vs 8%, respectively, P = 0.12).

In both arms, as reported by patients received crocin and placebo, the most common toxicities were grade 1 (except nausea) included increased appetite (14.7%; 2.9%), sedation (8.8%; 5.8%) headache (8.8%; 5.8%), nausea (8.8%; 2.9%), hypomania (5.8% each), stomach ache (5.8%; 2.9%), vomiting (2.9%; 2.9%), and swelling of feet (2.9%; 0%), respectively.

4. Discussion and conclusion

Our study had some limitations. The final results of the present study demonstrate that the duration of intervention seems to be short and long-term interventions might lead to better effects of crocin on measured parameters of neuropathy. Furthermore, we did not evaluate the effects of crocin on biomarkers of inflammation, oxidative stress, and its related gene expression. Since crocin efficacy was prominent in CIPN patients, lack of a positive control agent is noticeable in our study. Although, as we mentioned before, there are controversial and contradictory reports about the efficacy of the chemical agents like antidepressants, lamotrigine, gabapentin etc. in the prevention and treatment of CIPN.

Due to inadequate efficacy, severe adverse/side effects, and the possible drug-drug interaction with the chemotherapy regimens of currently available options, there is no advantageous agent used for relieving CIPN and subsequent pains. Given the relatively high incidence of CIPN, this represents an urgent unmet medical need. The development of a well-tolerated nonopioid oral agent for effective therapy of CIPN is a priority for oncology patients. The main mechanisms by which cause CIPN includes immune mediated mechanisms, alterations in the function of neuronal ion channels, degeneration of axons in myelinated neurons, altered calcium homeostasis within the cell, and oxidative stress by the induction of reactive oxygen species (Han and Smith, 2013). Our results indicate that no significant difference was found between the two groups (C/P and P/C arms) at baseline regarding age, gender, type of cancer, chemotherapy protocol cycle, and chemotherapy drug (s). So, it looks like that the groups were compatible for these confounding factors, and these factors cannot affect the obtained results. Based on our findings, crocin has beneficial effects on CIPN and neuropathic pain due to chemotherapy injections. Primary and secondary outcomes indicate that CIPN-lowering effects of crocin were gradually potentiated during both sections of the trial in both C/P and P/C arms. Crocin exerted its maximal efficacy significantly and predominantly in the last three weeks of each section of the trial. Perhaps, if we continued crocin therapy, even the symptoms of neuropathy would disappear and the patients would recover completely. These results might indicate the possible cumulative effects of herbs such as crocin (Schloss et al., 2017). Crocin-induced adverse effects were mild, and no treatment-induced intolerable, debilitating effects were observed in both groups. In the following, the closest studies in line with ours are mentioned. With regard to the main mechanisms of CIPN, the below studies can confirm the neuroprotective effects of crocin and/or saffron against CIPN. Saffron diminishes extracellular glutamate and aspartate (the main neuroexcitatory transmitters involved in pain signaling and neuropathy) in the hippocampus of anaesthetized rats (Hosseinzadeh et al., 2008). Saffron extract (30 mg/kg) and its main constitute crocin (30 mg/kg) alleviate mechanical allodynia and thermal hyperalgesia in a rat model of chronic constriction injury (CCI, the most used experimental model of peripheral nerve injury equivalent to clinical neuropathic pain) (Bennett and Xie, 1988) for up to 40 days after surgery (Safakhah et al., 2016), indicating that these substances display therapeutic effect in the management of neuropathic pain (Safakhah et al., 2016; Amin and Hosseinzadeh, 2012). Intracerebroventricular administration of crocin (6 μg/5 μL) significantly decreased both thermal hyperalgesia and mechanical allodynia and also, peripheral injection of this agent at the dose of 60 mg/kg significantly decreased mechanical allodynia at day 14 after the induction of CCI in rats (Vafaei et al., 2020). Central or peripheral administration of Win 55-212-2 (cannabinoid receptor agonist) or AM 251 (cannabinoid receptor antagonist) modulates the analgesic effect of crocin indicating that analgesic effects of crocin probably are mediated by an endocannabinoid mechanism (Vafaei et al., 2020). Ethanolic and aqueous extracts of saffron can provide neuroprotection through attenuation of oxidative stress, inflammation and apoptosis in the CCI rat model (Amin et al., 2014). Even, preemptive administration of crocin at a lower dose (15 mg/kg) can maintain the analgesic effect of morphine in CCI rats indicating the synergistic effects between the 2 compounds (Safakhah et al., 2020). In another study, it has been shown that 10 mg/kg of ethanolic extract of saffron displayed anti-nociceptive activity during both acute and chronic phases of formalin test and also confined inflammation 30%, 66%, and 80% with doses of 2.5, 5, and 10 mg/kg, respectively, whereas these effects were prominent with crocin administration in the chronic phase of formalin test in mice (Nasri et al., 2011). Since opioid receptor antagonist (naloxone), NMDA receptor antagonist (dextromethorphan), and nitric oxide synthase inhibitor (L-NAME) reverse the analgesic activity of saffron extract during the 2 phases of formalin test, it seems that saffron and its constituents exert these neuroprotective effects against pain signaling via probable stimulation of opioid receptors, NMDA glutamatergic and nitric oxide dependent pathways (Nasri et al., 2011). Crocin (30 mg/kg) shows neuroprotective effects against peripheral neuropathy induced by streptozotocin injection in diabetic rats and it looks like this effect could be associated with its anti-hyperglycemic and antioxidant properties (Farshid and Tamaddonfard, 2015). A four-week treatment period with crocin (100 and 200 mg/kg/day) prevents the induction of hyperalgesia in diabetic mice (Hosseinzdeh et al., 2009). Crocin reduces endoplasmic reticulum stress, inflammatory gene expression, and the expression of endoplasmic reticulum stress genes XBP-1/s in the spinal cord on day 7 post-experimental autoimmune encephalopathy induction (Deslauriers et al., 2011). Crocin reduces neuropathology in experimental autoimmune encephalomyelitis (EAE) through the prevention of syncytin-1- induced astrocyte and oligodendrocyte cytotoxicity (syncytin-1 has been contributed to oligodendrocyte death and neuroinflammation) (Christensen, 2005; Antony et al., 2004). The aqueous extract of saffron (50, 100 and 200 mg/kg) inhibited diazinon (organophosphorus pesticide)-induced increase of inflammation, oxidative stress and neuronal damage biomarkers (Moallem et al., 2014). Crocin remarkably increases the activity of antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) and reduces malondialdehyde (MDA, as an index of lipid peroxidation) level in the ischemic cortex in a rat model of ischemic stroke (Vakili et al., 2014). Saffron extract improved the lipid peroxidation status and monoamine oxidase (MAO-A, MAO-B) activity in the whole brain and cerebellum of aluminum exposed mice (Linardaki et al., 2013).

Pretreatment with saffron (5 and 25 mg/ml) and crocin (10 and 50 μM) decrease reactive oxygen species-mediated high glucose-induced neuronal death (toxicity) in PC12 cells (Mousavi et al., 2010).

In summary, findings of our study suggest a benefit to crocin use to treat CIPN-related symptoms. Analysis of pain scores measured serially in the current clinical trial reveals a significant and gradual decline in the severity of CIPN-related pain in all patients who completed the remedy protocol correctly. In addition to recent clinical studies on saffron about its anxiolytic (Milajerdi et al., 2018) antidepressant (Talaei et al., 2015), and memory-enhancing effects (Akhondzadeh et al., 2010) (closest trials about the saffron effects on the nervous system), the present study also confirms the neuroprotective properties of this natural agent indicating its promising effect in patients suffering from CIPN and/or other types of neuropathy. So, complementary studies in order to investigate the exact mechanisms of neuroprotective effects of saffron and/or crocin in CIPN seem to be necessary and will be very helpful to affirm our findings.

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CRediT authorship contribution statement

Hooman Bozorgi: Project administration, Supervision, Writing – original draft, Methodology, Software, Formal analysis, Data curation, Writing – review & editing, The project administrator, Hooman Bozorgi has supervised the project and took the lead in writing the article, designed the method, implemented the software, and contributed to statistical analysis and data curation. **Farahnaz Ghahremanfard:** Conceptualization, Writing – review & editing, has contributed to conceptualization and provision of study materials/patients and carried out experiments. **Ehsan Motaghi:** Formal analysis, Writing – review & editing, has contributed to statistical analysis and revision of the manuscript. **Maryam Zamaemifard:** Writing – review & editing, has contributed to provision of study materials/patients and carried out experiments. **Melika Zamani:** Writing – review & editing, has contributed to provision of study materials/patients. **Amin Izadi:** Writing – review & editing, has contributed to provision of study materials/patients.

Declaration of competing interest

The authors declared there is no conflict of interest related to this article.

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