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L-Carnosine protects against Oxaliplatin-induced peripheral neuropathy in colorectal cancer patients: A perspective on targeting Nrf-2 and NF-κB pathways

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ABSTRACT

Background: Chemotherapy-induced peripheral neuropathy is a common side effect afflicting cancer patients treated with oxalipatin based chemotherapy.

Aim: The study investigated the potential prophylactic effect of L-carnosine against acute oxaliplatin neurotoxicity in colorectal cancer patients with emphasis on the redox (Nrf-2, MDA), inflammatory (NF-κB, TNF-α), and apoptotic (caspase-3) parameters.

Methods: In this pilot study, 65 patients were recruited using a prospective randomized controlled study design and enrolled randomly into two arms; Arm A, 31 patients received FOLFOX-6 regimen (oxaliplatin, 5FU & leucovorin) and Arm B, 34 patients received FOLFOX-6 regimen and daily oral L-carnosine (500 mg) along the treatment period. Patients were followed up for three months, then both arms were analyzed for neuropathy incidence/grade and any additional toxicities according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTC version 4).

Results: The neuropathy grading evaluation of Arm B *vs* Arm A revealed that 17 patients (56.7%) *vs* 11 patients (35.5%) suffered grade 1, one patient (3.3%) *vs* 19 patients (61.3%) suffered grade 2, while 12 patients (40%) *vs* one patient (3.2%) were normal.

In arm B, the addition of L-carnosine decreased significantly the levels/activity of NF-κB (27%) and TNF-α (36.6%); this anti-inflammatory effect entailed also its anti-oxidative and anti-apoptotic effects, thus MDA level (51.8%) and caspase-3 activity (49%) were also reduced, whereas Nrf-2 was increased (38.7%) as compared to Arm A.

In both arms a significant correlation was only evident between TNF-α and the neuropathy grading score ($P < .03$); the correlation analysis was significantly positive between NF-κB and both Nrf-2 and caspase 3.

Conclusion: L-Carnosine exerted a neuroprotective effect against oxaliplatin-induced peripheral neuropathy in colorectal cancer patients by targeting Nrf-2 and NF-κB pathways.

1. Introduction

Albeit the efficacy of platinum analogs in cancer treatment, serious side effects, especially peripheral sensory neurotoxicity, often prevent their administration at their full efficacious doses. This may therefore delay their curative rate and may considerably affect the patients quality of life (Argyriou et al., 2012a; Cavaletti, 2014). Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect afflicting patients with cancer treated with neurotoxic chemotherapeutic agents and is defined as inflammation, injury, or degeneration of the peripheral nerve fibers (Hilkens and ven den Bent, 1997).

Oxaliplatin, a platinum derivative, is an alkylating agent used in many types of cancers. Following intracellular hydrolysis, the platinum compound binds to DNA forming cross-links that inhibit DNA replication and transcription, resulting in cell death, considering that cytotoxicity is cell-cycle nonspecific (Lévi et al., 2000). Oxaliplatin, as a widely used third-generation platinum analog approved for use in the treatment of metastatic colon cancer, is reported by the Food and Drug

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Administration to be responsible for > 70% rate of symptomatic neurotoxicity with any severity (Ibrahim et al., 2004) and often leads to treatment discontinuation (McWhinney et al., 2009; Grothey and Goldberg, 2004). In other studies, approximately 80% of colorectal cancer patients treated with oxaliplatin alone or in combination with other chemotherapeutics experienced neurotoxicity (Argyriou et al., 2012b, 2013) and impairment may be permanent.

The current knowledge of pathophysiological mechanisms of Oxaliplatin-induced peripheral neuropathy (OIPN) is substantial and recent advances in this area could lead to the development of some novel therapeutic strategies. The axis of pathophysiological factors responsible for OIPN converge at three of the most extensively studied pathways, the oxidative stress, inflammatory, and apoptotic pathways. Regarding the redox pathway, it is associated with the elevation of malondialdehyde and the suppression of nuclear factor-2 erythroid related factor-2 (Nrf-2), while the inflammatory cascade includes the upregulation of the transcriptional factor nuclear factor-kappa light chain enhancer of B cells (NF-κB) and its downstream molecule, tumor necrosis factor-alpha (TNF-α). Besides, the apoptotic signaling pathway can be assessed by the increased apoptotic markers including caspase-3 (Negi et al., 2011; Li et al., 2008).

So far, several agents hold promise for their ability to prevent OIPN with some other adverse actions, including acetylcysteine, amifostine, calcium and magnesium infusions, diethyldithiocarbamate, glutathione, Org 2766, venlafaxine, and oxycarbazepine (Avan et al., 2015).

Fortunately, nutraceuticals including natural and herbal products proved to open new horizons for promising add-on therapeutics in a plethora of diseases. Carnosine, an endogenous dipeptide composed of β-alanine and L-histidine, occurs naturally in some species of vertebrates, including humans. The highest concentration is observed in skeletal muscle tissue, the stomach, the kidney, cardiac muscle, the brain and olfactory bulb (Garibala and Sinclair, 2000; Hill et al., 2007). Studies performed on the gastric-nemius muscle show that carnosine content in the human body is dependent on gender (the level is higher in males), age (there is a decrease in carnosine concentration with age) and diet (organisms on a vegetarian diet have a lower concentration of carnosine in the skeletal muscles) (Everaert et al., 2011; Derave et al., 2010). In addition, researchers suggest that synthesis of carnosine in muscles is dependent on the availability of the amino acid alanine in the body (Hill et al., 2007; Artioli et al., 2010).

When provided as a supplement to humans, carnosine possesses antioxidant properties, acting against free radicals, which are factors contributing to the aging human systems. Carnosine scavenges both reactive oxygen and nitrogen species and creates complex chemical compounds with zinc and copper ions (Guney et al., 2006; Hipkiss, 2009). Carnosine dipeptide may inhibit lipid peroxidation through a combination of free radical scavenging and metal chelation activity. It also provides cells with an antioxidant system that functions in the cytosolic environment, where water soluble oxidation mediators are often present in high concentrations (Garibala and Sinclair, 2000). Previous studies highlighted the potential neuroprotective ability of carnosine as a supportive treatment against neurotoxins (Kozan et al., 2008), effect that can be mediated by virtue of its antioxidants and anti-inflammatory effects (Hipkiss, 2009; Shiheit et al., 2010; Cheng et al., 2011).

The aim of the current study is to assess the potential prophylactic effect of exogenous antioxidant “L-carnosine” on oxaliplatin-induced peripheral neuropathy in colorectal cancer patients and to assess the possible role of the Nrf-2 and NF-κB pathways.

2. Patients and methods

2.1. Subjects

Prior to initiation, this study received approval by the Ethical Committee of the Faculty of Pharmacy at Cairo University (PT 1451). The study enrolled 65 Egyptian patients, who accepted to sign the informed consent and were suffering from colorectal cancer in the oncology department at El-Demerdash hospital, Ain Shams University. The 65 patients were primarily recruited, whereas 4 patients (6.2%) dropped from the study, so the actual sample was 61 patients including 29 males (47.5%) and 32 females (52.5%). The mean age of the patients was 45.8 ± 9.6 years with the youngest age is 24 and the oldest is 65 years.

Table 1
Patients' demographics and biochemical markers in both arms, A and B at baseline.

Groups Variable	Arm A (n = 31)	Arm B (n = 30)
Age (years)	45.9 ± 8.6	45.6 ± 10.5*
Male/female (N)	13/18 (41.9% / 58.1%)	16/14** (53.3% / 46.7%)
Scr (mg/dl)	0.8 ± 0.3	1 ± 0.2*
BUN (mIU/ml)	13 ± 5	15 ± 3*
AST (U/L)	27 ± 10	30 ± 12*
ALT (U/L)	26 ± 11	25 ± 10*
Total serum bilirubin (mg/dl)	0.4 ± 0.1	0.6 ± 0.3*
Hb (gm/dl)	14 ± 2	13 ± 2*
Platelet ($\times 10^3$/ul)	325 ± 100	300 ± 90*
TLC ($\times 10^3$/ul)	7.5 ± 3	5.5 ± 2*

Arm A: Patients taking FOLFOX-6 only, Arm B: Patients taking FOLFOX-6 & L-carnosine as an add on. Values are means ± SD. Statistical tests used: * Independent sample t-test and **Chi- square test. SCr: serum creatinine; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Hb: hemoglobin; TLC: total leucocyte count.

Table 2
Effect of L-carnosine on the development of neuropathy grade in both Arms (A& B) according to NCICTCAE-v4 scoring system.

Neuropathy grade Groups	Normal	Grade 1	Grade 2
Arm A	1 (3.2%)	11 (35.5%)	19 (61.3%)
Arm B	12 (40%)*	17 (56.7%)*	1 (3.3%)*

Arm A: 31 patients taking FOLFOX-6 only, Arm B: 30 patients taking FOLFOX-6 & L-carnosine as an add on. Values are presented as number of patients and the corresponding percentage values are shown in parenthesis. Statistical test used is Chi- square test, (*) significance from Arm A at $P < .05$.

Inclusion criteria was based on a thorough history taking and a clinical and pathological examination. Patients were considered eligible if they meet the following criteria;

- Adult males and females with age $^{>}$18 and $^{<}$65.
- Patients having colorectal cancer treated with FOLFOX-6 regimen (oxaliplatin, 5FU & leucovorin) for the first time.

On the other hand, patients with the following criteria were excluded from this study; those suffering from diabetes mellitus, peripheral neuropathy because of any other disease or drug, severe renal impairment (CrCl $^{<}$ 30 ml/min), epilepsy, and those taking vitamin B. Also excluded from the study were patients who previously took oxaliplatin or any other chemotherapeutic agent known to cause peripheral neuropathy, those taking antidepressants or mono-amine oxidase inhibitors (MAOIs), non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, opiates or any other analgesics or pain killers. Pregnant or lactating patients were not recruited.

The patients were divided into 2 arms as per protocol, where the number of patients randomized into Arm A (FOLFOX-6 regimen) were 31(50.8%) and Arm B 30 (49.2%) patients receiving FOLFOX-6 regimen and oral L-carnosine (500 mg/day, (Hipkiss, 2005)). Both treatments were given all along the three months treatment duration. FOLFOX-6 was given every 2–3 weeks for 3 months, dose depending on the

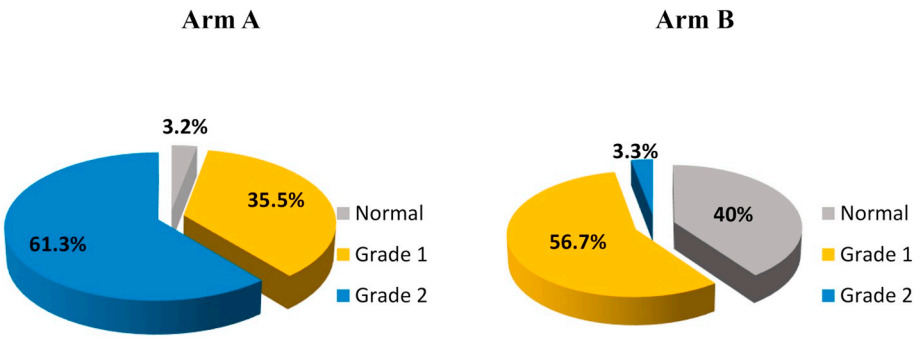

Fig. 1. Arm A: 31 patients taking FOLFOX-6 ONLY, Arm B: 30 patients taking FOLFOX-6 & L-carnosine as an add on. The relative frequencies of neuropathy grade in Arm A and Arm B presented as pie chart; statistical analysis was carried out using Chi square test.

Peripheral neuropathy classification according to NCI-CTC V4 (US Department of Health and Human Services, 2009):

Grade 1: Asymptomatic; loss of deep tendon reflexes or paresthesia.

Grade 2: Moderate symptoms; limiting instrumental ADL.

Grade 3: Severe symptoms; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Table 3
Paired analysis of oxidative/inflammatory/apoptotic markers before and after treatment among patients in arm A and arm B.

Groups Variable	Arm A		Arm B	
	Baseline	3 months after treatment	Baseline	3 months after treatment
Nrf-2 (pg/ml)	1097.30 ± 341.10	751.60 ± 269.80*	1272.10 ± 368.5	1225.93 ± 348.86
MDA (nmol/ml)	1.01 ± 1.37	1.37 ± 0.57*	1.15 ± 0.350	0.66 ± 0.24*
NF-κB (U/L)	1446.90 ± 871.12	1696.00 ± 1083.60*	1774.5 ± 721.6	1236.5 ± 572.80*
TNF-α (ng/ml)	0.03461 ± 0.00974	0.03248 ± 0.00754	0.04 ± 0.010	0.02 ± 0.01*
Caspase-3 (ng/ml)	16.08 ± 4.50	23.80 ± 6.90*	13.60 ± 5.02	10.90 ± 5.64*

Arm A: 31 patients taking FOLFOX-6 only, Arm B: 30 patients taking FOLFOX-6 & L-carnosine as an add on. Values are presented as means ± SD, (*) significant difference from baseline values, at $P < .05$, using paired *t*-test. Nrf-2:human nuclear factor erythroid 2-related factor 2, MDA: malondialdehyde, NF-κB: human nuclear factor-kappa B, TNF-α: Tumor necrosis factor-alpha.

patient's BSA, whereas L-carnosine, as an add on treatment, was administered orally once daily for 3 months, starting the day before oxaliplain.

2.2. Neuropathy assessment

Neuropathy grade was evaluated according to NCI-CTCAE-v4 which is widely accepted throughout the oncology community as the standard classification and severity grading scale for adverse events in cancer therapy clinical trials and other oncology settings (US Department of Health and Human Services, 2009).

2.3. Blood sampling and biochemical analyses

Venous blood samples were obtained from all patients before treatments and 3 months later. Samples were allowed to clot and sera were then separated by centrifugation at 3500 rpm/20 min/25 °C and stored into 2 aliquots at −80 °C until assessment of the biochemical parameters.

Commercial human ELISA kits (Glory Science Co. (Del Rio, TX, USA) were used for the determination of NF-κB (Cat.# 11358) (Perkins, 2007), Nrf-2 (Cat.# F4554) (Kubben et al., 2016), TNF-α (Cat.#50111) (Harrington et al., 2008), and caspase-3 (Cat.# C1681) (Taylor, 2001). Lipid peroxide was measured as malondi-aldehyde (MDA) using colorimetric kit (Bio-diagnostic, Diagnostic and Research Reagents, Cairo, Egypt) (Ohkawa et al., 1979).

Usual biochemical laboratory tests, such as complete blood count, as well as liver and kidney function tests were assessed before patients receive their chemotherapy cycle then every two weeks throughout the treatment period.

2.4. Statistical analysis

Statistical analysis was carried out using SPSS v22.0 IBM statistical package for social sciences & Microsoft Office 2016 and Graphpad prism 5. Categorical data were subjected to descriptive analysis using frequency and percentage, while for the scale data, mean and standard deviation (SD) were used. Tests for inferential statistics and correlation were Chi Square test, independent *t*-test, simple paired *t*-test and Pearson correlation test. Moreover, ANOVA test was used to compare means of scale variables *vs* categorical data, followed by Scheffe's test. The significance level was set at $P < .05$.

3. Results

3.1. Basal demographic data and biochemical values in both arms

The actual sample in this study was 61 patients consisting of 29 males (47.5%) and 32 females (52.5%) with an age range from 24 to 65 and mean age of 45.8 ± 9.6 years. The demographic factors are presented in Table 1, which shows no significant differences between both arms regarding age, gender, male/female ratios, as well as the basal values of the laboratory data; *viz.,* Scr, BUN, AST, ALT, total serum bilirubin, Hb, platelet, TLC. The absence of significance between the two arms confirms the randomization process.

3.2. Comparison between neuropathy grades in both arms

L-Carnosine alleviated the symptoms of neuropathy in colorectal cancer patients resulting from FOLFOX-6 treatment. There was a lowering in the number of patients suffering from neuropathy and in the grade of neuropathic pain produced by treatment. Table 2 and Fig. 1 depict the evaluation of neuropathy grading according to NCI-CTCAE-v4 scoring system. There was a highly statistical significant difference between the 2 arms, where in Arm B the number of patients suffering from grade 1 neuropathy was 17 patients (56.7%), while only 1 patient suffered from grade 2 (3.3%) and 12 patients (40%) were considered normal. Contrastingly, in arm A the number of patients suffering from grade 1 were 11 patients (35.5%), while 19 (61.3%) suffered from grade 2 neuropathy, and only one patient (3.2%) remained normal.

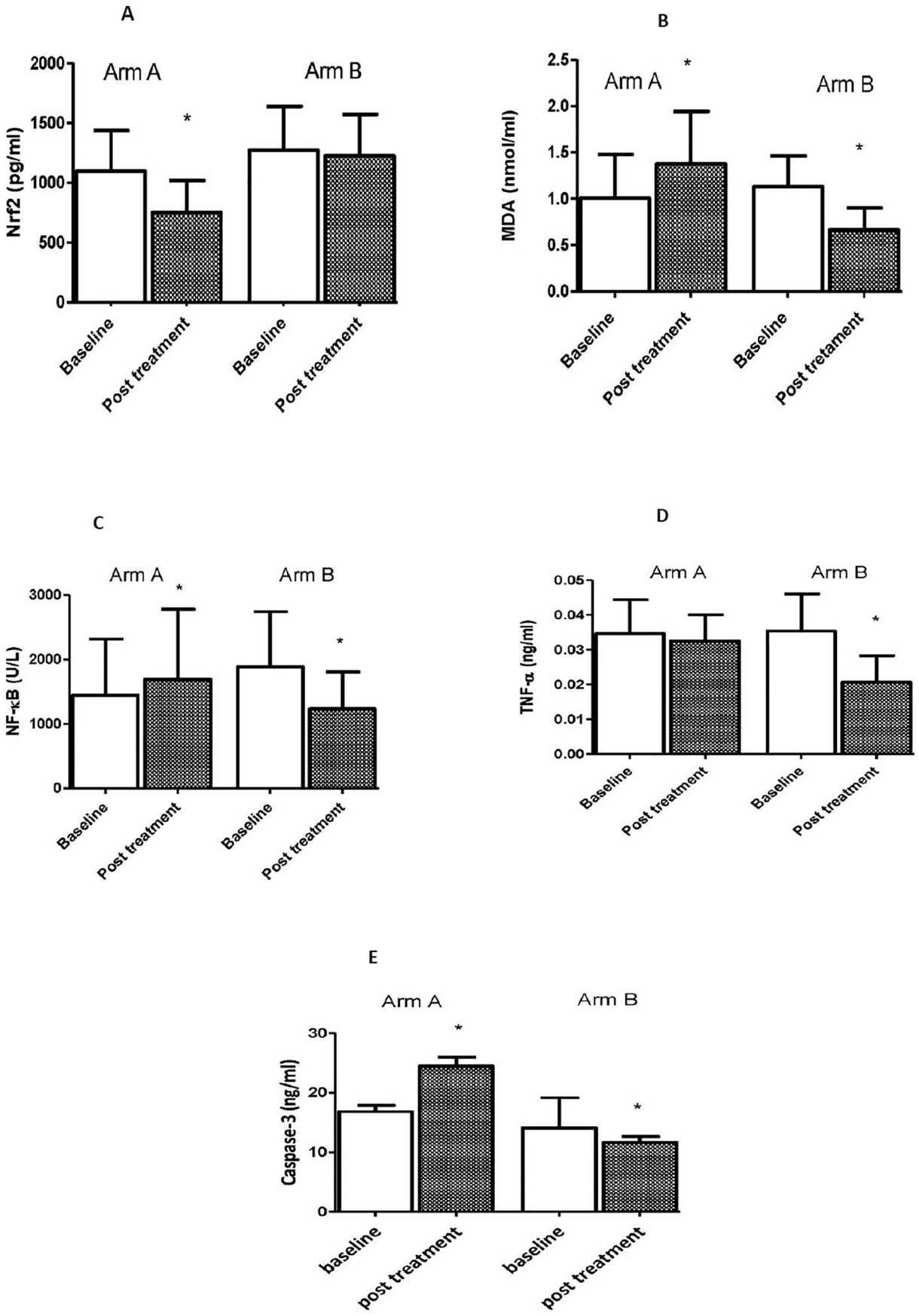

Fig. 2. Changes in the values of the assessed oxidative/inflammatory/apoptotic biomarkers pre- and post-treatments. Arm A: 31 patients taking FOLFOX-6 only, Arm B: 30 patients taking FOLFOX-6 & L-carnosine as an add on. Values are given as mean ± S.D., statistical significance was tested using paired *t*-test. Significant at $P < 0.05$. A), (*) significant difference from baseline values, at $P < .05$ human nuclear factor erythroid 2-related factor 2 (Nrf2); B) Malondialdehyde (MDA); C) human nuclear factor-kappa B (NF-κB), D) Tumor necrosis factor-alpha (TNF-α); E) human caspase 3 (Caspase-3).

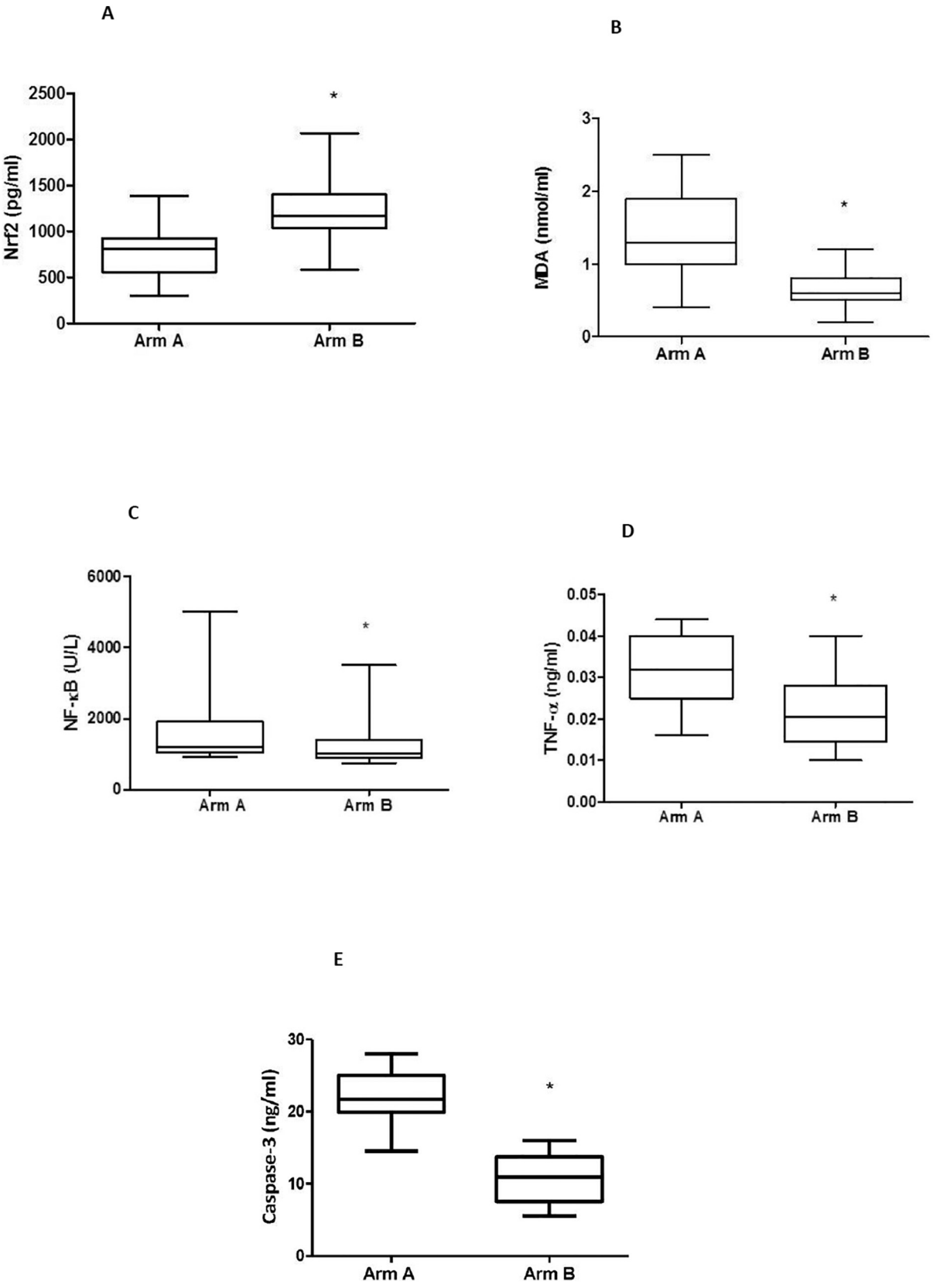

Fig. 3. The impact of L-carnosine on oxidative, inflammatory and apoptotic markers in serum of patients with colorectal cancer. Arm A: 31 patients taking FOLFOX-6 only, Arm B: 30 patients taking FOLFOX-6 & L-Carnosine as an add on. Values are given as median and range using box plot graph, Significant at $P < 0.05$. A), (*) significant difference from arm A A) Human nuclear factor erythroid 2-related factor 2 (Nrf2), B) Malondialdehyde (MDA), C) human nuclear factor-kappa B (NF-κB), D) Tumor necrosis factor-alpha (TNF-α), E) human caspase 3 (Caspase-3).

Table 4
Comparison between both Arms (A&B) regarding post treatment laboratory results and their respective clinical scoring system.

Variable		Normal values	Pathological values		
			Mild	Moderate	Severe
Scr (mg/dl) Values ± SD		0.9 ± 0.3	1.6 ± 0.2	–	–
Number of patients	Arm A	30	1	–	–
	Arm B	29	1	–	–
BUN (mg/dl) Values ± SD		15 ± 4	30	–	–
Number of patients	Arm A	30	1	–	–
	Arm B	30	0	–	–
AST (IU/L)		35 ± 5	60 ± 4	130	
Number of patients	Arm A	27	3	1	–
	Arm B	24	6	–	–
ALT (IU/L)		30 ± 5	70 ± 5	150 ± 10	–
Number of patients	Arm A	29	1	1	–
	Arm B	27	2	1	–
Total serum bilirubin (mg/dl)		0.7 ± 0.4	1.5 ± 0.1	–	-
Number of patients	Arm A	29	2	–	–
	Arm B	30	–	–	–
Hb (g/dl)		15 ± 1	11 ± 1	9 ± 1	7 ± 0.5
Number of patients	Arm A	9	11	10	1
	Arm B	16	6	6	2
Platelet ($\times 10^3$/μl)		250 ± 100	100 ± 25	–	–
Number of patients	Arm A	26	5	–	–
	Arm B	24	6	–	–
TLC ($\times 10^3$/μl)		7 ± 3	3.5 ± 0.4	2.5 ± 0.4	1.5 ± 0.4
Number of patients	Arm A	16	7	7	1
	Arm B	17	7	3	3

Arm A: Patients taking FOLFOX-6 only, Arm B: Patients taking FOLFOX-6 & L-carnosine as an add-on. Values given are means ± SD for post treatment laboratory results, their respective clinical scoring system as well as the corresponding number of patients. Statistical test used is the Chi square test at $P < .05$. Mild, moderate, and severe are grades of toxicity according to NCICTCAE-v4. SCr: serum creatinine; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Hb: hemoglobin; TLC: total leucocyte count.

Table 5
Correlation between the neuropathy grading and the assessed oxidative/inflammatory/apoptotic biomarkers post-treatment among patients receiving FOLFOX-6 alone.

Post treatment markers	Grade of neuropathy	N	Mean ± SD
Nrf-2 (pg/ml)	0	1	972.29
	1	11	750.44 ± 192.07
	2	19	740.63 ± 312.96
	Total	31	751.59 ± 269.75
MDA (nmol/ml)	0	1	1.3
	1	11	1.21 ± 0.55
	2	19	1.47 ± 0.58
	Total	31	1.37 ± 0.56
NF-κB (U/L)	0	1	975.00
	1	11	1695.91 ± 922.25
	2	19	1733.95 ± 1205.81
	Total	31	1695.97 ± 1083.58
TNF-α (ng/ml)	0	1	0.040
	1	11	0.034 ± 0.006
	2	19	0.031 ± 0.008
	Total	31	0.032 ± 0.008
Caspase3 (ng/ml)	0	1	17.09
	1	11	24.14 ± 7.79
	2	19	24.05 ± 6.63
	Total	31	23.84 ± 6.94

Significance was tested at $P < .05$ using ANOVA test. N: Number of patients. Nrf-2: human nuclear factor erythroid 2-related factor 2; MDA: Malondialdehyde; NF-κB: human nuclear factor-kappa B, TNF-α: Tumor necrosis factor-alpha.

Table 6
Relation between neuropathy grading and assessed parameters post treatment in patients taking FOLFOX-6 and L-carnosine as an add on therapy.

Post treatment markers	Grade of neuropathy	N	Mean ± SD
Nrf-2 (pg/ml)	0	12	1187.04 ± 388.14
	1	17	1243.09 ± 337.11
	2	1	1400.78
	Total	30	1225.92 ± 348.85
MDA (nmol/ml)	0	12	0.73 ± 0.28
	1	171	0.60 ± 0.2
	2	1	0.90
	Total	30	0.66 ± 0.24
NF-κB (U/L)	0	12	1445.42 ± 790.89
	1	17	1055.88 ± 278.04
	2	1	1800.00
	Total	30	1236.50 ± 572.75
TNF-α (ng/ml)	0	12	0.02 ± 0.007
	1	17	0.02 ± 0.007
	2	1	0.04*
	Total	30	0.02 ± 0.01
Caspase-3 (ng/ml)	0	12	13.27 ± 7.57
	1	17	9.21 ± 3.32
	2	1	11.67
	Total	30	10.92 ± 5.64

*. Significance was tested at $P < .05$ using ANOVA test, followed by Scheffe's test. (*) As compared to grade of neuropathy.
N: Number of patients. Nrf-2: human nuclear factor erythroid 2-related factor 2; MDA: Malondialdehyde; NF-κB: human nuclear factor-kappa B, TNF-α: Tumor necrosis factor-alpha.

3.3. Changes in the values of the assessed parameters pre- and post-treatment

Table 3 and Fig. 2 present the paired analysis test used to analyze the effect of the three months therapy on the studied variables in both arms compared to their baseline values (before treatment). Regarding Arm A, FOLFOX-6 treatment for three months leveled off Nrf-2 significantly by 31.5%, whereas it augmented MDA significantly by 35.6%. NF-κB by 17.2%, and caspase-3 activity by 48%. The change in TNF-α was subtle yet statistically insignificant, as it decreased by 5.8%.

Table 7
Correlation between the biochemical parameters post treatment in both groups.

	Nrf2 post (pg/ml)	TNF-α post (ng/ml)	Caspase3 post (ng/ml)	MDA post (nmol/ml)
NF-κB (U/L)	Arm A $r = 0.37^{*}$ $P = .04$ Arm B $r = 0.09$ $P = .63$	Arm A $r = 0.26$ $P = .16$ Arm B $r = -0.016$ $P = .408$	Arm A $r = 0.48^{**}$ $P = .01$ Arm B $r = 0.51^{**}$ $P = .004$	Arm A r = 0.31 $P = .09$ Arm B $r = 0.36^{*}$ $P = .05$
Nrf2 (pg/ml)		Arm A $r = 0.49^{**}$ $P = .01$ Arm B $r = -0.02$ $P = .93$	Arm A $r = 0.33$ $P = .07$ Arm B $r = -0.37^{*}$ $P = .045$	Arm A $r = 0.15$ $P = .42$ Arm B $r = -0.15$ $P = .45$
TNF-α (ng/ml)			Arm A $r = -0.014$ $P = .942$ Arm B $r = 0.001$ $P = .995$	Arm A $r = 0.31$ $P = .09$ Arm B $r = -0.20$ $P = .29$
Caspase3 (ng/ml)				Arm A $r = 0.01$ $P = .98$ Arm B $r = 0.24$ $P = .204$

(*) and (**) Significant at $P \leq .05$ and 0.01 (2-tailed) using Pearson Correlation.

Regarding Arm B and after three months of administration of L-carnosine, Nrf-2 was slightly decreased by 3.67%, but did not reach a significant level, however L-carnosine significantly dampened MDA level by 42.6%, NF-κB by 30.3%, TNF-α by almost the half (47.3%) and caspase-3 by 18.4%.

3.4. Differences between the post-treatment effects with and without L-carnosine on biochemical markers

Table 3 and Fig. 3 present the post-treatment effect of L-carnosine on oxidative stress (Nrf-2 & MDA), inflammatory (NF-κB & TNF-α), and apoptotic (caspase-3) markers of patients with colorectal cancer. The results signify the statistical improving effect of L-carnosine in Arm B patients *vs* those of Arm A. The daily oral administration of L-carnosine improved the redox balance, where it improved the anti-oxidant capacity by elevating Nrf-2 level (63%), while halved the MDA level (51.8%), compared to Arm A. This improving effect entailed the inflammatory and apoptotic markers as well, where L-carnosine abated NF-κB (27%) and TNF- α (36.3%), as well as caspase-3 (54.2%), as compared to Arm A.

3.5. Comparison between post treatment laboratory results in both arms

Table 4 shows post treatment laboratory results concerning kidney function tests, liver function tests, and haematological tests in both Arms (A&B) and their respective clinical scoring system. All variables did not show any statistical significant difference between both arms, indicating that L-carnosine did not induce any toxic effects as shownin Table 4.

3.6. Relation between the neuropathy grading and assessed parameters post treatment in both arms

Table 5 shows that in all patients taking FOLFOX-6 only, as the neuropathy grade is elevated there was no coinciding changes in oxidative, inflammatory, and apoptotic markers. Similarly, in patients taking FOLFOX-6 and L-carnosine as an add on, neuropathy was not accompanied by changes in the parameters assessed, except TNF-α where it was double the level at grade 2 compared to normal ($p < .03$) as shown in Table 6.

3.7. Correlation between the assessed parameters in both arms

Correlation among the various assessed parameters in colorectal cancer patients on treatment with FOLFOX-6 alone or in combination with L-carnosine is shown in Table 7 and Fig.4.

4. Discussion

Oxaliplatin, in combination with 5FU, leucovorin, capecitabine, and/or irinotecan are common chemotherapy regimens for colorectal cancer that confer significant clinical benefit in terms of progression free survival and response rates (Adam et al., 2010; de Gramont et al., 2000). However, the most common toxicity associated with Oxaliplatin treatment is chemotherapy-induced peripheral neuropathy (CIPN) (Grothey, 2010; Argyriou et al., 2014).

To the best of the authors' knowledge, this is the first study to confirm the ability of L-carnosine to alleviate OIPN in colorectal cancer patients, and to shed light on some the possible mechanism of action. To fulfill this, the first goal was assessed by comparing the neuropathy grade in Arm A (convential treatment) and (Arm B supplemental L-carnosine + anticancer treatment) according to NCI-CTC version 4. Additionally, the study verified the potential mechanism of L-carnosine *via* comparing serum levels of the redox (Nrf-2 & MDA), inflammatory (NF-κB & TNF-α) and pro-apoptotic (Caspase-3) markers between the two arms.

Intriguingly, positive results were obtained when L-carnosine was added to the basic therapy FOLFOX-6 regimen (oxaliplatin, 5FU & leucovorin) of patients with colorectal cancer. L-Carnosine protected 40% of patients in Arm B from peripheral neuropathy, compared to only 3% of patients in Arm A with no neuropathy. It also dampened peripheral neuropathy such that 57% of patients in Arm B developed grade I, and only 3% were in grade II, compared to Arm A, in which 36% were in grade I and 61% developed grade II neuropathy. Previously, L-Carnosine improved neurological functions in a mouse model of focal cerebral ischemia (Min et al., 2008), in a Parkinson's like disorder (Tsai et al., 2010), in zinc-induced neuropathy (Mizuno et al., 2015), and improved cognitive function in patients with Parkinson's disease (Hipkiss, 2015).

The axis of pathophysiological factors responsible for OIPN converge at three of the most extensively studied pathways; *viz.*, oxidative stress, inflammation, and apoptosis. Regarding the oxidative stress pathway, the current study showed that a considerable improvement in the anti-oxidant capacity was observed in patients receiving L-Carnosine in addition to their traditional therapy. In Arm B, L-carnosine elevated Nrf-2 by 38.7% compared to Arm A, although both arms had a comparable Nrf-2 mean serum levels at their base line (1210.14 pg/ml and 1097.26 pg/ml, respectively). The carnosine-mediated anti-oxidant effect entails the lipid peroxidation biomarker MDA. In Arm B, as well, the MDA level was halved (p value = .001) by the end of the treatment period, despite the comparable level between the two arms (1.15 nmol/ml *vs* 1.01 nmol/ml) at the beginning of the study. These results signify the role of L-carnosine in correcting the redox imbalance, where the

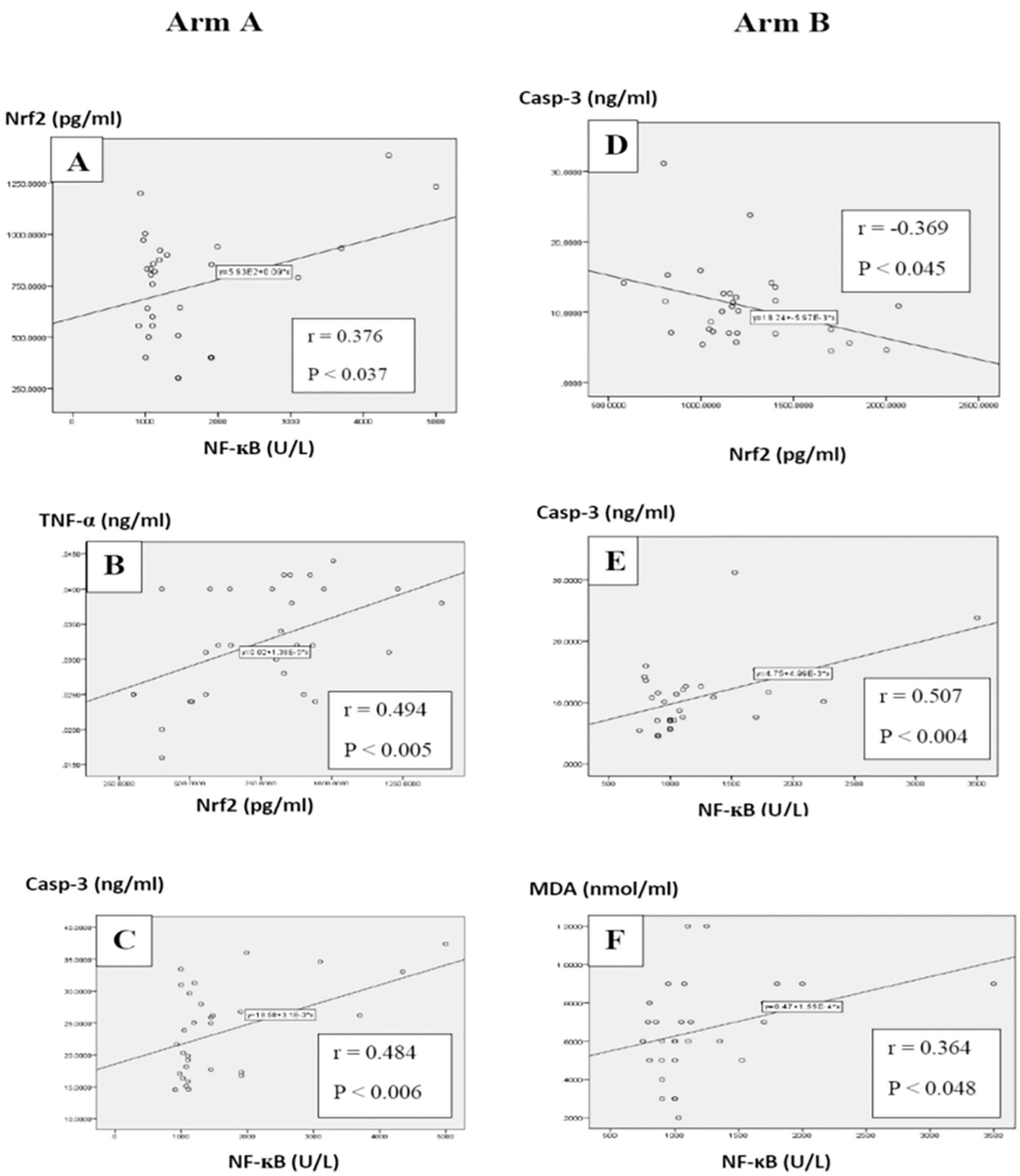

Fig. 4. Correlation among the various assessed parameters in colorectal cancer patients on treatment with FOLFOX-6 alone or in combination with L-carnosine for three months. In Arm A patients, the following correlations were detected, a positive correlation between (A) Nrf2 and NF-κB; (B) TNF-α and Nrf2, and (C) Casp-3 and NF-κB. In Arm B patients, the following correlations were detected, (D) a negative correlation between Casp-3 and Nrf2, but a positive correlation was seen between (E) Casp-3 and NF-κB, as well as (F) MDA and NF-κB. Nrf2: Nrf-2: nuclear factor erythroid 2-related factor 2; MDA: malondialdehyde; NF-κB: nuclear factor-kappa B, TNF-α: tumor necrosis factor-alpha; Casp-3: caspase 3. Statistical analysis was carried out using the Pearson correlation test and the data are presented as scatter dot plot. Arm A: 31 patients taking FOLFOX-6; Arm B: 30 patients taking FOLFOX-6 & L-carnosine as an add-on.

enhancement of the nuclear transcriptional factor Nrf-2 is known to orchestrate the anti-oxidant machinary. When translocated into the nucleus, Nrf-2 tethers with the antioxidant response element of specific genes that encode the antioxidant molecules in order to preserve the redox homeostasis (Gupte et al., 2013).

In alignment with our findings, ample data highlighted the anti-oxidant capacity of L-carnosine in different oxidative stress-associated diseases in patients with diabetes (Babizhayev et al., 2015), open-angle glaucoma (Babizhayev, 2012), and Parkinson's-like model (Tsai et al., 2010). Moreover, in an *in vitro* study, carnosine, but not other conventional antioxidants, protected neurons against MDA-mediated protein cross-linking, mitochondrial dysfunction, and the activation of ROS-dependent MAPK signaling pathway (Cheng et al., 2011). Our findings were also consistent with the results of the previous study of (Baky et al. 2016), in which carnosine alone or with cyclosporine A down-modulated the head injury-induced oxidative stress markers.

Besides modulating the redox balance, L-carnosine had an impact on the inflammatory markers, where inclusion of L-carnosine (Arm B) significantly (*P* value = .04) abated the serum levels of NF-κB (27%), whose baseline mean values were not significantly different from that in Arm A. Since NF-κB, as a transcriptional factor, is responsible for the transcription/formation of several pro-inflammatory mediators, including TNF-α, our data showed that the serum level of the pro-inflammatory surrogate marker, TNF-α was reduced in Arm B (36.6%, *P* value = .001) compared to Arm A, though all patients had the same level at the beginning of the study (0.035 ng/ml and 0.034 ng/ml, respectively). It is noteworthy mentioning that in a vicious cycle, TNF-α can also stimulate NF-κB. The L-carnosine effect may be attributed to the elevation in Nrf-2; a crosstalk between the two transcriptional factors studied herein; *viz.*, Nrf-2 and NF-κB is documented. In pathological states, NF-κB abrogates the anti-oxidant beneficial effect of Nrf-2 (Liu et al., 2008), whereas, it was found lately that elevated level of Nrf-2 mediate an anti-inflammatory effect, beside the anti-oxidant one. Nrf-2 can inhibit NF-κB and its downstream molecules as TNF-α, either by preventing the ubiquitination of the inhibitory kappa B (I-κB)- α, hence suppressing the effect of NF-κB or by enhancing the hemeoxygenase (HO)-1 that inhibits TNF-α-dependent activation of NF-κB (Yerra et al., 2013).

Moreover, it is reported that increased NF-κB causes neuroinflammation along with other inflammatory mediators released by the activated astrocytes and microglia, thus, the ability of L-carnosine to protect against OIPN can be in part due to the increased Nrf2 with its antioxidant machinery and the inhibition of both NF-κB and TNF-α. This can be supported by the findings of Ralhan et al. (2009), who reported that Nrf-2 activators inhibit NF-κB activity, thus affording dual protection from chemotherapy- induced damage to various organs. Our results are in harmony with that of Baky et al. (2016), where treatment of traumatized rats with carnosine and/or cyclosporine A, significantly decreased serum levels of TNF-α compared with untreated traumatized ones. Moreover, similar results were obtained from previous studies that showed reduced TNF- α levels after oral administration of carnosine in animal models of brain damage and diabetes mellitus (Lee et al., 2005).

The Nrf2 nuclear transcription factor also regulates the expression and induction of a battery of genes encoding cytoprotection in response to chemical and radiation stress. This leads to reduced apoptosis, enhanced cell survival, and increased drug resistance, accordingly Nrf2 has a role in up-regulation of anti-apoptotic protein Bcl-2 and contributes to stress induced apoptosis and cell survival (Niture and Jaiswal, 2012). Similarly NF-κB is a transcription factor for a large group of genes, which are involved in several different pathways. For instance, NF-κB activates groups of pro-apoptotic and anti-apoptotic genes (Danial and Korsmeyer, 2004).

Thus the ameliorative anti-oxidant and anti-inflammatory effects of L-carnosine were also reflected on its anti-apoptotic effect, where L-carnosine in Arm B, halved the chemotherapy and ROS-induced apoptosis (49%) (*P* value = .001) assessed by serum caspase-3; again the two arms had almost similar baseline values (13.6 ng/ml and 16.08 ng/ml). This effect corroborates with the results of Cheng et al. (2011), who found that carnosine favored cell survival by sparing the anti-apoptotic marker Bcl-2 and suppressing the apoptotic marker Bax, associated with a decreased ratio of Bcl-2/Bax (Cheng et al., 2011). Similarly, in the trial conducted by Baky et al. (2015), the anti-apoptotic effect of carnosine, assessed by the inactivation of caspase-3 can play a role in minimizing the secondary traumatic brain injury and improving the neurological outcome (Baky et al., 2016). Moreover, considerable studies have showed that carnosine provides an anti-apoptotic role in the animal models of hypoxia-ischemia brain damage and subarachnoid hemorrhage-induced early brain injury through lowering the expression of caspase-3 protein (Wang et al., 2013; Zhang et al., 2015). Again, the anti-apoptotic effect can partially be linked with the enhanced Nrf2 and the suppressed NF- κB.

The current study also analyzed the effect of the three months therapy on the studied variables in both arms compared to baseline values. Regarding Arm B, carnosine improved most of the deleterious pathological effects resulting from chemotherapy after three months of treatment. NF-κB significantly decreased by 30.3% and consequently TNF-α declined to the half (50%) compared to the baseline values. Although, the carnosine antioxidant effect caused a subtle insignificant increase in Nrf-2 (3.6%), but it markedly decreased lipid peroxidation as it reduced MDA significantly (42.6%). The apoptotic marker, caspase-3, was also significantly lowered by 20%.

L-carnosine is a safe compound, where it does not cause addiction nor accumulates in the body after long term consumption because of its cleavage by the carnosinase enzyme into easily excreted amino acids that are from the organism (Boldyrev, 2009). This fact was confirmed in the present study, where the intake of carnosine for three months did not produce any undesirable effects on the consuming patients as indicated by the laboratory variables (ALT, AST, total serum bilirubin, Scr, BUN, Hb, platelets, TLC), which were assessed at baseline and before each cycle of chemotherapy and did not show any statistical significant difference between both arms.

In conclusion, the dietary supplementation with L-carnosine proved to improve OIPN by amelioration of the pathophysiological triad of inflammation, oxidative stress and apoptosis. These results led to the recommendation of the safe add-on therapy of carnosine to chemotherapeutic agents, especially those associated with CIPN, and opens thereby, a new supportive strategy in oncologic diseases.

Data statement

Prior to initiation, this study received approval by the Ethical Committee of the Faculty of Pharmacy at Cairo University (PT 1451).

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