

Carvone Attenuates Irinotecan-Induced Intestinal Mucositis and Diarrhea in Mice

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Abstract

Intestinal mucositis is referring to inflammatory or ulcerative lesions of the oral or gastrointestinal tract; one of the main reasons is treatment with cancer chemotherapy. The prodrug Irinotecan is converted by carboxylesterase to the active metabolite SN-38, conjugated by UGT enzyme to SN-38G and then deconjugated by β -glucuronidase produced by intestinal bacterial flora to produce SN-38. Irinotecan induces intestinal mucositis and diarrhea due to increased concentration of its active metabolite (SN-38). To evaluate the protective effect of carvone, I.P injection of (75mg/kg/day) of irinotecan for 4 days to induce intestinal mucositis, carvone administered to mice orally for 6 days starting from day 1. Results showed that carvone (50mg/Kg and 100mg/Kg) significantly and by dose-dependent manner attenuated body weight loss (-9.39±1.56 vs. -23.21±1.65 %), diarrhea scores (0.50±0.244 vs. 2.67±0.211) and serum TNF- α level (1361.44±55.075 vs. 3402.12±321.56 ng/ml) compared to experimental model group. In conclusion, carvone exerted a dose dependent anti-inflammatory and protective effect by attenuation irinotecan-induced intestinal mucositis.

Keywords: Intestinal mucositis, Irinotecan, Carvone, Body weight variation, Diarrhea score, TNF α level.

الكارفون يثبط التهاب المعوي والإسهال المستحث بواسطة الأيرينوتيكان في الفئران

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الخلاصة

يشير التهاب الغشاء المخاطي المعوي إلى الآفات الالتهابية أو التقرحية في الفم أو الجهاز الهضمي؛ أحد الأسباب الرئيسية هو جرعة عالية من العلاج الكيميائي Irinotecan. هو دواء أولي يتم تحويله إلى مستقلب نشط SN-38 بواسطة carboxylesterase، مترافق بواسطة إنزيم UGT إلى SN-38G ثم يتم تفكيكه بواسطة β -glucuronidase الذي تنتجه البكتيريا الموجودة بصورة طبيعية بالأمعاء لإنتاج SN-38 يتسبب Irinotecan في التهاب الغشاء المخاطي المعوي والإسهال بسبب زيادة تركيز SN-38 المعوي ويؤدي إلى زيادة الإجهاد التأكسدي. لدراسة التأثير الوقائي للكارفون في irinotecan- يسبب التهاب الغشاء المخاطي، التهاب الغشاء المخاطي المعوي الناتج عن حقن (75 IP مجم / كجم / يوم) لمدة 4 أيام. أظهرت النتائج أن الكارفون الذي تم تناوله عن طريق الفم للفئران لمدة 6 أيام بدءاً من اليوم الأول، أدى إلى انخفاض كبير في فقدان وزن الجسم (-9.39 ± 1.56 مقابل -23.21 ± 1.65)، ودرجة الإسهال (0.50 ± 0.244 مقابل 2.67 ± 0.211)، ومستوى TNF α في البلازما (1361.44 ± 55.075 مقابل 3402.12 ± 321.56) مقارنة بمجموعة التحكم في النموذج. في الختام، كارفون له تأثير وقائي في التهاب الغشاء المخاطي والإسهال الناتج عن الأيرينوتيكان.

الكلمات المفتاحية: التهاب الغشاء المخاطي المعوي، إرينوتيكان، كارفون، تغير وزن الجسم، درجة الإسهال، عامل نخر الورم الفا

Introduction

Mucositis is an inflammatory or ulcerative lesions of the oral or gastrointestinal tract. Infectious disease, immune deficiency and Medications as with high-dose cancer therapy could be considered as one of main causative factors for mucositis ⁽¹⁾. The condition was developed by breakdown of the mucosal barrier leading to: significant ulceration of the oral cavity and GIT leading to passing of bacteria into the systemic circulation, increasing the risk of infections, an imbalance between secretion and absorption, demonstrated as diarrhea⁽²⁾. Chemotherapy induced diarrhea (CID) being a principal reason for pain and reduced quality of life during cancer treatment because it is one of the most debilitating adverse effects of chemotherapy ⁽³⁾. The progression of mucositis can be divided into five

phases: initialization, activation, signal amplification, ulceration and Healing phase⁽⁴⁾. Severe diarrhea leading to dehydration, renal insufficiency, malnutrition, fever, neutropenia, infectious complications, or serious electrolyte imbalances can result in hospitalization ⁽⁵⁾. The presence of CID can impact on providers to modulate chemotherapeutic agents, decrease treatment doses, delay therapy, or even cease therapy, resulting in potentially worsened clinical outcomes⁽⁵⁾. Irinotecan hydrochloride is a prodrug that is metabolically activated to 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterase 2 in the blood and liver, and thereafter SN-38 is conjugated in the liver by uridine diphosphate glucuronosyl transferase 1A1 to produce a β -glucuronide conjugate, SN-38G⁽⁶⁾.

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Irinotecan and SN-38 are a potent inhibitor of DNA topoisomerase I, a fundamental enzyme that controls DNA structural alterations by relaxing DNA supercoil as well as relegating cleaved DNA strands throughout DNA transcription and replication⁽⁶⁾. Two types of diarrhea induced by irinotecan can be distinguished: early and late-onset diarrhea. Early-onset diarrhea begin during, or immediately after, drug infusion and is occur due to increased cholinergic activity, which triggers intestinal contractility and diminish the absorptive capacity of the mucosa⁽⁷⁾ Late-onset diarrhea appear approximately 8–10 days after irinotecan infusion and is recognized by a more severe course, which is probably occur due to damage of the intestinal mucosa as a result of increased oxidative stress by biliary-secreted or intestinally deconjugated SN-38⁽⁸⁾. The treatment of CID involves non pharmacologic and pharmacologic interventions to inhibit diarrhea and careful serial estimation to abolish significant volume depletion or comorbidities that would need particular intervention or hospitalization⁽⁹⁾. Anti-diarrheal therapies used are: Opioid, Octreotide, Budesonide, Intestinal Alkalinization, Alteration of Intestinal Micro flora [Prebiotic and Probiotic, Antibiotics], D-saccharic Acid 1,4-Lactone (SAL) and Glutamine. Different plants extract found to have a protective effect against irinotecan-induced intestinal mucositis and diarrhea, as *Kampo Kampo* (Japanese \Chinese herbal) and *Mentha spicata L.* (spearmint) the plant that we will study its assumed protective effect against CID. The main components of mentha spicata oil were carvone (40.8% ± 1.23%) and limonene (20.8% ± 1.12%), followed by 1,8-cineole (17.0% ± 0.60%), β-pinene (2.2% ± 0.25%), cis-dihydrocarvone (1.9% ± 0.49%), and dihydrocarveol (1.7% ± 0.31%)⁽¹⁰⁾. In term of biological uses, *mentha spicata* used as antispasmodics and anti-platelets and insecticides⁽¹¹⁾.

Carvone (p-mentha-6, 8-dien- 2-one) is a chiral monoterpene ketone that can be achieved by distillation. It occurs naturally in two enantiomer forms: 1. S (+)- carvone (the major component in the essential oil of caraway [*Carum carvi*]. 2. R(-)-carvone (which is existing in the essential oil of spearmint [*Mentha spicata*]⁽¹²⁾ (R)-(-)-Carvone gives ant nociceptive activity⁽¹³⁾. Both (R)-(-)-carvone and (S)-(+)-carvone decreased peripheral nerve activity, likely through the blockade of voltage-gated sodium channels⁽¹²⁾. carvone has an obvious role as it has been reported to exert anticancer effect⁽¹⁴⁾, it was manifests various possible biological activities namely antioxidant, antimicrobial, nematocidal and antidiabetic effect⁽¹⁵⁾.

Materials and Methods

Chemicals and drugs

Irinotecan vial (100mg/5ml) was purchased from Kocak pharma, Turkey. R-(-) carvone was purchased from Sigma, USA. TNF-α ELISA kit purchased from Shanghai, China.

Preparation of stock solution of carvone

A stock solution of carvone was prepared by dissolving 0.2 ml of carvone in 10 ml of corn oil then the solution is mixed by vortex to obtain a final concentration of (1.5 mg/0.1 ml) according to the carvone dose for mice (50mg/Kg)⁽¹⁶⁾. Another stock solution of carvone was prepared by dissolving 0.4 ml of carvone in 10 ml of corn oil then the solution is mixed by vortex to obtain a final concentration of (3 mg/0.1 ml) according to the carvone dose for mice (100mg/kg)⁽¹⁶⁾.

Experimental protocol

Twenty -four albino female mice weighing (25-50 g) were brought from Animal House of College of Pharmacy, Baghdad University. Animals were maintained on standard conditions of temperature, humidity, and light /dark cycle. They had free access to standard diet and water. Mice were divided into 4 groups, each group with 6 mice as following and illustrated in figure 1:

Group I: six animals received a single oral daily dose of 0.1ml of normal saline for 7 successive days starting day 1. This group served as a normal control group.

Group II: six animals received a single dose of intraperitoneal irinotecan (75mg/kg) for 4 successive days starting day 1. This group served as a model control group.

Group III: six animals received a single dose of intraperitoneal irinotecan (75mg/kg) for 4 successive days starting day 1 with 0.1ml of carvone of dose 50mg/kg by oral gavage for 6 successive days starting day 1.

Group IV: six animals received a single dose of intraperitoneal irinotecan (75mg/kg) for 4 successive days starting day 1 with 0.1ml of carvone of dose 100mg/kg by oral gavage for 6 successive days starting day 1.

Administration of carvone done by oral gavage at 8AM and 8PM daily from day 1 through day 6. Euthinazation has done on day 7 by diethyl ether followed by cervical dislocation.

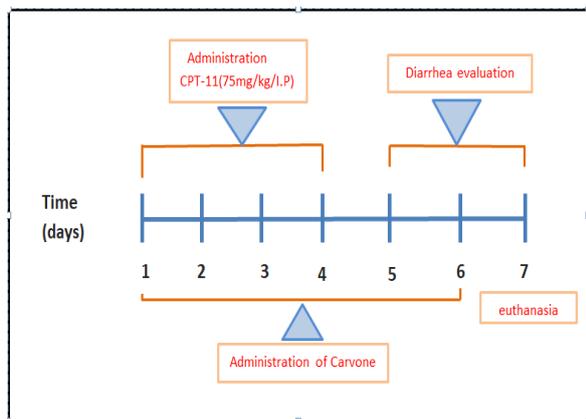


Figure1. Schematic representation of the experimental model design.

Serum collection: On day 7, about 0.5ml of blood was drawn from retro-orbital area and collected in eppendorf tube and let to completely coagulated, then centrifuged for 15 min. at 5000 rpm in a cold centrifuge at 4 °C. The serum was then collected and froze for later use to measure TNF- α .

Body weight measurement

Mice body weights were recorded at 8AM daily. Weight values were expressed as a percentage of weight variation in relation to the baseline weight⁽¹⁷⁾.

$$\text{Weight variation (\%)} = \{(W2-W1)/W1\} * 100$$

W1= Baseline mouse weight
W2 = weight of mouse during the study period.

Diarrhea score measurement

The diarrhea scores were estimated daily starting 24 hr. after the last irinotecan dose between day 5 and 7. The intensity of delayed-onset diarrhea was recorded at 8:00 AM daily after the last irinotecan dose till euthanization (figure 1). Diarrhea was estimated using standard reference scale ⁽¹⁸⁾, as shown in table1.

Table 1. Diarrhea score reference scale. ⁽¹⁸⁾

Extent of diarrhea	Manifestations	score
Normal	Normal stool or absent	0
Slight	Slightly wet and soft stool	1
Moderate	Wet and unformed stool with moderate perianal staining of the coat	2
Severe	Watery stool with severe perianal staining of the coat	3

Determination of Serum TNF- α Level

The principle of TNF α determination in serum is based on enzyme-linked immunosorbent assay (ELISA) that was use the sandwich- ELISA technique. The micro ELISA plate has been pre-coated with antibody specific to mouse TNF α ⁽¹⁹⁾.

Statistical analysis

Values expressed as mean \pm standard error mean (SEM). Mice body weight values presented as weight variation percent \pm SEM. All statistical analyses were carried out using the statistical package of social science (SPSS) software version 25. Differences were considered significant at $P < 0.05$. Intergroup comparisons were made by Student's t-test.

Results

Effect of carvone on serum TNF- α level

The data presented in table 2 and figure 2 showed that administration of irinotecan in the model control group (group II) resulted in a significant increase in inflammation ($P < 0.05$) compared to the normal control group (group I) resulted in TNF- α serum levels elevation from 3402.12 \pm 321.56pg/dL to 858.7 \pm 47.79pg/dL respectively.

Administration of Carvone 50mg/Kg (group III), and carvone 100mg/Kg (group IV), produce a significant ($P < 0.05$) reduction in serum TNF α levels (2210.48 \pm 188.29pg/dL, 1361.45 \pm 55.07pg/dL) respectively compared to model group (group II, 3402.12 \pm 321.56pg/dL) as shown in table 2 and figure 2. This interesting result indicates a dose-dependent anti-inflammatory effect of carvone and a counteracting role in irinotecan-induced mucositis.

Table 2. Effects of carvone on serum TNF α level in irinotecan- induce intestinal mucositis in mice

Groups	Type of treatment	TNF α (pg/dL)
I	Normal saline	858.70 \pm 47.79
II	Irinotecan	3402.12 \pm 321.56 *
III	Carvone 50mg/kg +irinotecan	2210.48 \pm 188.29 *
IV	Carvone 100mg/kg +irinotecan	1361.44 \pm 55.075 *

Data are expressed as mean \pm standard error of means (SEM).

(*) Significantly different ($P < 0.05$).

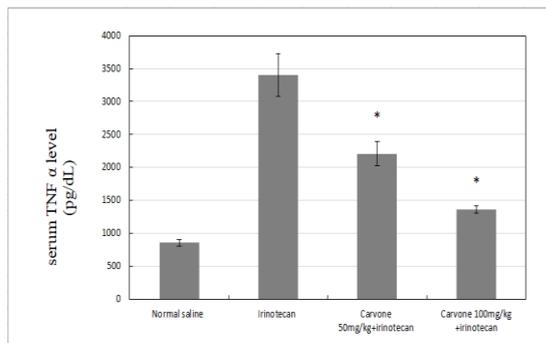


Figure 2. Effect of carvone on serum TNF-α level in irinotecan-induced intestinal mucositis. (*) denotes significant difference ($P<0.05$) compared with experimental model group (group II).

Effect of carvone on mice body weight

Analysis of mice weight variation percent revealed that significant ($P<0.05$) weight loss started from Day 2 in all groups except non-treated control

group (Figure 3). Mice received irinotecan only (group II) showed significant ($P<0.05$) weight loss from Day 2 till euthanization. On day 7, a maximum weight loss variation percent recorded of $(-23.21\pm1.65\%)$. On the other hand, treatment with carvone (50mg/Kg) significant ($P<0.05$) attenuated weight loss compared to model group on day 7, where the weight variation percent calculated was $(-13.78\pm1.62\%)$. Furthermore by increasing the dose, treatment with carvone (100mg/Kg) significantly ($P<0.05$) hampered weight loss on days 5, 6 and 7 compared to experimental model. Interestingly, on day 7 the weight variation percent calculated was $(-9.39\pm1.56\%)$. Analysis of the data of weight variation percent clearly revealed that carvone exerted a dose-dependent attenuation of weight loss induced by irinotecan (Figure 3).

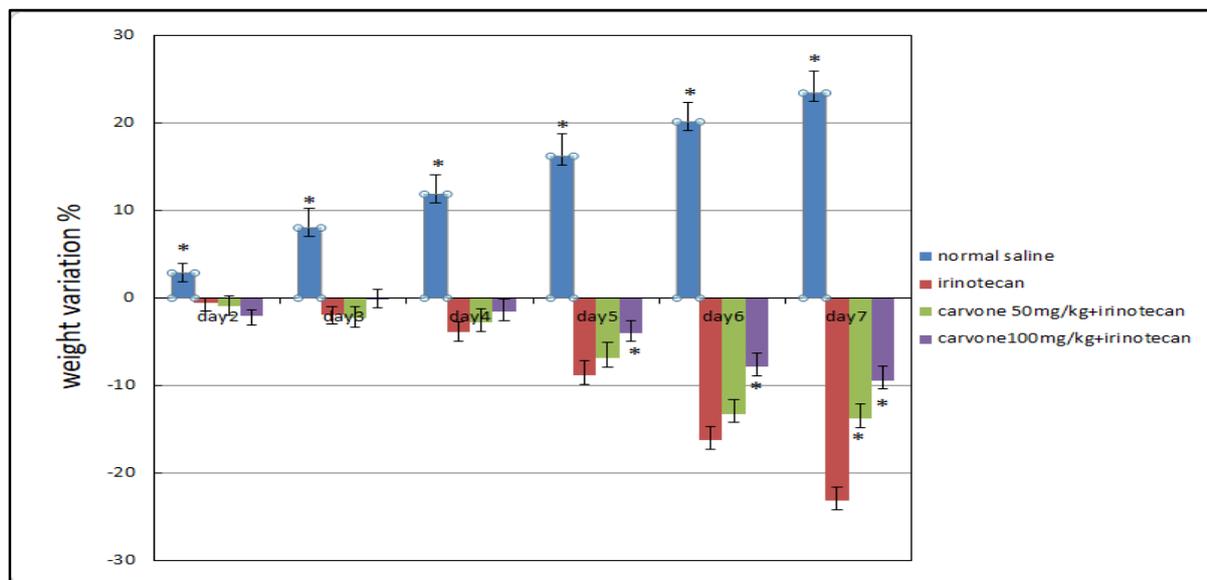


Figure 3. Effect of carvone treatment on mice body weight variation in irinotecan-induced intestinal mucositis and diarrhea. (*) denotes significant difference ($P<0.05$) compared with experimental model group (group II).

Effect of carvone on diarrhea score

Data obtained from the present study showed that diarrhea scores began to rise on day 5 in the model control group (group II) and other treatment groups 24 hours after the last irinotecan injection as shown in table 4. On day 7, mice in model control group (group II) suffered from severe diarrhea as manifested from the significant ($P<0.05$)

high diarrhea score (2.67 ± 0.211) compared to the normal control group (0). However, treatment with carvone (50mg/kg) resulted in significant ($P<0.05$) attenuation of the diarrhea scores (1.00 ± 0.258) compared to model group. Increasing the dose of carvone to (100mg/kg) resulted in significant ($P<0.05$) and more reduction in diarrhea scores (0.05 ± 0.244) compared to model group.

Table 4. Effect of carvone treatment on diarrhea score in irinotecan-induced intestinal mucositis.

Group	Treatment groups	Diarrhea score (Mean \pm SEM)			
		D4	D5	D6	D7
I	Normal saline	0	0	0	0
II	Irinotecan	0	0.17 \pm 0.167	1.00 \pm 0	2.67 \pm 0.211
III	Carvone 50mg/kg \pm irinotecan	0	0	.83 \pm 0.167	1.00 \pm 0.258 *
IV	Carvone 100mg/kg \pm irinotecan	0	0	0	0.50 \pm 0.244 *

(*): significant difference ($P < 0.05$).

(D): days of treatment.

Discussion

Mucositis is referring to inflammatory or ulcerative lesions of the oral or gastrointestinal tract⁽¹⁾. Not only it is associated with an adverse symptoms which include pain, vomiting and diarrhea but it may limit patients' ability to tolerate treatment if not adequately prevented and managed⁽²⁰⁾. Chemotherapy-induced mucositis being a principal reason for pain and reduced quality of life during cancer treatment⁽³⁾. Irinotecan induce intestinal mucositis and late onset diarrhea, The pathogenesis of this toxic side effect is that irinotecan and its active metabolite, SN-38, can cause direct damage to the intestinal mucosal cells, leading to DNA damage and generation of reactive oxygen species due to oxidative stress⁽⁴⁾. Furthermore irinotecan can stimulate several signaling pathways, such as the (NF)- κ B, increase the level of the inflammatory markers such as tumor necrosis factor (TNF α) and interleukin (IL)-1 β and elevate the expression of inflammation related factor such as prostaglandin E₂ (PGE₂)⁽²¹⁾.

In the present study, results showed a significant increase in TNF α serum level in mice confirmed irinotecan-induced intestinal mucositis mediated by inflammation. a previous study by (Odilia Antunes Fernandes, Simone. 2018) confirmed that TNF α plays a crucial role in mucositis⁽²²⁾, moreover, this study showed significant decrease in mice body weight with increase in diarrhea score. This result confirmed by other study and explained by lowering in intestinal absorptive capacity lead to diarrheal episodes, fluid loss and body weight loss⁽²³⁾.

TNF α serum level results showed significant decrease during the use of carvone at dose 100mg/kg rather than the other dose of carvone (50mg/kg), this effect may be due to increasing the dose, carvone has antioxidant and anti-inflammatory effect⁽²⁴⁾ leading to scavenging of ROS, decrease oxidative stress, inhibition of pro-inflammatory cytokines and decrease inflammation. The successful reduction in serum TNF α in mice treated with carvone confirmed by significant reduction in mice body weight loss

and diarrhea score, weight loss occur due to diarrhea and decreased food intake, carvone has antioxidant and anti-inflammatory effect leading to reduction in inflammation, mucositis and diarrhea. In addition to its antispasmodic effect⁽²⁵⁾ which leads to improvement of food intake and decrease diarrhea.

Conclusion

The present study results conclude that carvone exerted a dose dependent anti-inflammatory and protective effect by attenuation irinotecan-induced intestinal mucositis. Through reduction of inflammatory marker TNF- α and other observed parameters including weight loss and reduced diarrhea scores.

References

- Peterson DE, Bensadoun RJ, Roila F. Management of oral and gastrointestinal mucositis: ESMO clinical practice guidelines. *Ann Oncol.* 2011;22(SUPPL. 6):78–84.
- Van Seville YZA, Stansborough R, Wardill HR, Bateman E, Gibson RJ, Keefe DM. Management of Mucositis During Chemotherapy: From Pathophysiology to Pragmatic Therapeutics. *Curr Oncol Rep.* 2015;17(11).
- Skinner R. Nephrotoxicity of cancer treatment in children. *Ped Health.* 2010;4(5):519–38.
- Araújo RS, de Barros ALB. Intestinal Mucositis Induced by Chemotherapy: an Overview. *J Mol Pharm Org Process Res.* 2015;03(03):18–9.
- Benson AB, Ajani JA, Catalano RB, Engelking C, Kornblau SM, Martenson JA, et al. Recommended guidelines for the treatment of cancer treatment-induced diarrhea. *J Clin Oncol.* 2004;22(14):2918–26.
- Bao X, Wu J, Kim S, LoRusso P, Li J. Pharmacometabolomics Reveals Irinotecan Mechanism of Action in Cancer Patients. *J Clin Pharmacol.* 2019;59(1):20–34.
- Silber JH, Fridman M, Smith M, Simon R, Therapy C, Program E. CPT- 1-Induced Cholinergic Effects in Cancer Patients

- Ondansetron Compatible With Sodium Acetate. 2019;3–4.
8. Brandi G, Dabard J, Raibaud P, Di Battista M, Bridonneau C, Pisi AM, et al. Intestinal microflora and digestive toxicity of irinotecan in mice. *Clin Cancer Res.* 2006;12(4):1299–307.
 9. Stein A, Voigt W, Jordan K. Review: Chemotherapy-induced diarrhea: Pathophysiology, frequency and guideline-based management. *Ther Adv Med Oncol.* 2010;2(1):51–63.
 10. Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A, De Feo V. *Mentha spicata* essential oil: Chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of vibrio spp. strains. *Molecules.* 2015;20(8):14402–24.
 11. Abdul Jabbar AAS, Kathem SH. The protective effect of *Mentha spicata* ethanolic extract on irinotecan-induced mucositis in mice. *Iraqi J Pharm Sci.* 2019;28(1):37–43.
 12. Gonçalves JCR, Alves A de MH, de Araújo AE V., Cruz JS, Araújo DAM. Distinct effects of carvone analogues on the isolated nerve of rats. *Eur J Pharmacol.* 2010;645(1–3):108–12.
 13. Gonçalves JCR, Oliveira FDS, Benedito RB, De Sousa DP, De Almeida RN, De Araújo DAM. Antinociceptive activity of (-)-carvone: Evidence of association with decreased peripheral nerve excitability. *Biol Pharm Bull.* 2008;31(5):1017–20.
 14. Ding X, Chen H. Anticancer effects of Carvone in myeloma cells is mediated through the inhibition of p38 MAPK signalling pathway, apoptosis induction and inhibition of cell invasion. *J BUON.* 2018;23(3):747–51.
 15. Muruganathan U, Srinivasan S. Beneficial effect of carvone, a dietary monoterpene ameliorates hyperglycemia by regulating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. *Biomed Pharmacother.* 2016;84:1558–67.
 16. Muruganathan U, Srinivasan S, Indumathi D. Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats. *J Acute Dis.* 2013;2(4):310–5.
 17. Bastos CCC, Ávila PHM de, Filho EX dos S, Ávila RI de, Batista AC, Fonseca SG, et al. Use of *Bidens pilosa* L. (Asteraceae) and *Curcuma longa* L. (Zingiberaceae) to treat intestinal mucositis in mice: Toxicopharmacological evaluations. *Toxicol Reports.* 2016;3:279–87.
 18. Sakai H, Sagara A, Matsumoto K, Hasegawa S, Sato K, Nishizaki M, et al. 5-Fluorouracil Induces Diarrhea with Changes in the Expression of Inflammatory Cytokines and Aquaporins in Mouse Intestines. *PLoS One.* 2013;8(1):1–10.
 19. Zhang S, Garcia-D'Angeli A, Brennan JP, Huo Q. Predicting detection limits of enzyme-linked immunosorbent assay (ELISA) and bioanalytical techniques in general. *Analyst.* 2013;139(2):439–45.
 20. Sonis S, Elting L, Keefe D, Nguyen H, Grunberg S, Randolph-Jackson P, et al. Unanticipated frequency and consequences of regimen-related diarrhea in patients being treated with radiation or chemoradiation regimens for cancers of the head and neck or lung. *Support Care Cancer.* 2015;23(2):433–9.
 21. Ouyang M, Luo Z, Zhang W, Zhu D, Lu Y, Wu J, et al. Protective effect of curcumin against irinotecan-induced intestinal mucosal injury via attenuation of NF- κ B activation, oxidative stress and endoplasmic reticulum stress. *Int J Oncol.* 2019;54(4):1376–86.
 22. Odília Antunes Fernandes S. Repurposing the Thalidomide to the Treatment of Irinotecan-Induced Intestinal Mucositis: An Old Drug to a New Use. *Glob J Pharm Pharm Sci.* 2018;6(2).
 23. van Vliet MJ, Harmsen HJM, de Bont ESJM, Tissing WJE. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS Pathog.* 2010;6(5):1–7.
 24. Nogoceke FP, Barcaro IMR, de Sousa DP, Andreatini R. Antimanic-like effects of (R)-(-)-carvone and (S)-(+)-carvone in mice. *Neurosci Lett.* 2016;619:43–8.
 25. Souza FVM, Da Rocha MB, De Souza DP, Marçal RM. Carvone: Antispasmodic effect and mode of action. *Fitoterapia.* 2013;85(1):20–4.

