Chemico-Biological Interactions 291 (2018) 72–80

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Antineoplastic activity of mitomycin C formulated in nanoemulsions-based essential oils on HeLa cervical cancer cells

Mayson H. Alkhatib[a,*], Waad A. Al-Otaibi[a,b], Abdulwahab Noor Wali[c]

[a] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Chemistry Department, College of Science and Humanities, Shaqra University, Shagra, Saudi Arabia
[c] Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Keywords:
Cell culture
Cancer chemotherapy
Colloid
Nephelometry
Light-scattering
Fluorescence spectroscopy

ABSTRACT

Combining the essential oils (ESSOs) with the chemotherapeutic agent, mitomycin C (MMC), in nanoparticle can be beneficial in cancer therapy. The aim of the current study was to *in vitro* evaluate the antineoplastic effect of MMC, formulated in two different nanoemulsions (NE) based on two ESSOs, chamomile (Ch) and garlic (Gar), on HeLa cervical cancer cells. The z-average diameter of Ch-NE has slightly increased from 83.39 ± 12.85 nm to 91.18 ± 5.79 nm when mixed with MMC (Ch-MMC) whereas the z-average diameter of Gar-NE has markedly increased from 50.6 ± 1.96 nm to 75.64 ± 7.13 nm when loaded with MMC (Gar-MMC). The zeta potentials of both of Ch-NE and Ch-MMC, which were −1.91 ± 4.38 mV and −5.44 ± 5.26 mV, respectively, have differed from Gar-NE and Gar-MMC, which were 11.4 ± 2.29 mV and 11.5 ± 2.28 mV, respectively. Compared to MMC solution, the cell viabilities of HeLa cells, measured by the MTT assay, were reduced 42 and 20 times when subjected into Ch-MMC and Gar-MMC, respectively. The light microscopy images revealed that the cell membrane of the HeLa cells treated with Gar-NE or Gar-MMC were more altered relative to the cells treated with Ch-NE or Ch-MMC. In contrast, the nuclei of the HeLa cells, stained with DAPI and treated with Ch-NE or Ch-MMC, were more fragmented than the cells treated with Gar-NE or Gar-MMC, indicating that both of Ch-NE and Ch-MMC have passed the cell membrane and affected the nucleus directly whereas Gar-NE and Gar-MMC have got attached to the cell membrane causing damage to the cell. In conclusion, combining MMC with NE-based ESSOs has increased the cytotoxic effect of the MMC on the HeLa cells with different mechanism of actions.

1. Introduction

By 2030, global cancer mortality are increasing dramatically with a predicted 23.6 million new cases annually [1]. Among several types of cancers worldwide, cervical cancer is considered the fourth most common type among women [2]. Mitomycin C (MMC), a strong anti-tumor antibiotic [3], acts as an alkylating agent on the complementary DNA strand, forming crosslinks between neighboring guanine bases [4] and produces free radicals that may destroy the cancer cells [5]. Although MMC is a potent cytotoxic agent against a broad spectrum of human neoplastic diseases, its clinical use was enormously restricted because of its indiscriminate action on cancer and healthy cells [6]. Therefore, there is a need for novel formulations, which reduce the side effects caused by MMC and increase its therapeutic efficacy. Although MMC has been delivered in various types of nano- and micro-particles, it still fails to reach its target specifically due to the rapid elimination of the transporters in the body [7]. In order to conquer these limitations, a new method need to prepare MMC loaded ESSO- in-water NEs by a high pressure and heating technique, in which Tween 80 (T80) and Span 20 (S20) were employed to improve the ESSOs in water.

Chemoprevention of the ESSOs is the leading edge of cancer prevention, thereby reducing the incidence and mortality of cancer [8]. Previous studies have revealed that oxidative stress played a key role in the etiology of cancers [9]. ESSOs of chamomile (Ch) and garlic (Gar), natural anticancer and antioxidants, have been studied with respect to their protective effect of them and their capacity to scavenge free radicals damage that may lead to cancer [10–12]. In addition, Ch oil was found to be a potent polymerase antagonist relative to many other ESSOs and thereby has antitumor property [13]. Gar oil was reported as cell growth rate inhibitor by arresting the G2/M phase transition [14].

Abbreviations used: MMC, mitomycin C; ESSO, essential oil; Ch, chamomile; Gar, Garlic; NE, nanoemulsion; Ch-NE, chamomile-loaded NE; Ch-MMC, MMC-loaded NE-based chamomile oil; Gar-NE, garlic-loaded NE; Gar-MMC, MMC-loaded NE-based garlic oil; T80, Tween 80; S20, Span 20; MTT, (3(4,5dimethylthiazole- 2-yl)-2, 5 diphyneltetrazolium bromide); DAPI, 4′,6-diamidino-2-phenylindole; IC_{50}, maximal inhibitory concentration; NTU, nephelometric turbidity unit; ZP, Zeta potential; PDI, polydispersity index

* Corresponding author. Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia..
E-mail address: mhalkhatib@kau.edu.sa (M.H. Alkhatib).

https://doi.org/10.1016/j.cbi.2018.06.009
Received 7 March 2018; Received in revised form 22 May 2018; Accepted 11 June 2018
Available online 14 June 2018
0009-2797/

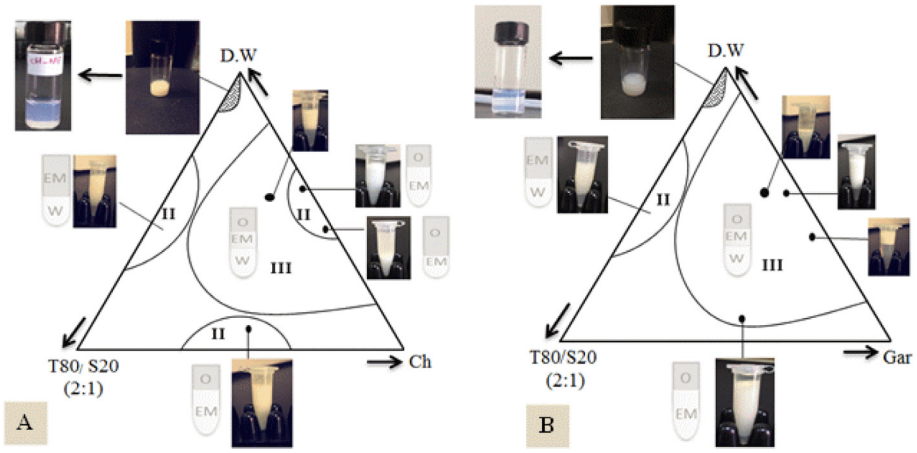

Fig. 1. Pseudo ternary phase diagrams formed by (A) chamomile (Ch) or (B) garlic (Gar) as the oil phase, distilled water (D.W) as the aqueous phase and the Tween 80/Span20 at 2:1 ratio, respectively.

Table 1
Physical properties of the NE-based ESSO.

NE formula	Z- average ± SD (nm)	ZP ± SD (mV)[a]	PDI[b]	pH
Gar-NE	50.6 ± 1.96	11.4 ± 2.29	0.03	4.05 ± 0.01
Gar-MMC	75.64 ± 7.13	11.5 ± 2.28	0.09	5.24 ± 0.03
Ch-NE	83.39 ± 12.85	−1.91 ± 4.38	0.15	4.02 ± 0.04
Ch-MMC	91.18 ± 5.79	−5.44 ± 5.26	0.06	4.29 ± 0.04

[a] ZP is the zeta potential; and.
[b] PDI is the polydispersity index.

Although Ch and Gar oils have shown promising anticancer activities and chemoprevention, one of their major limitations is the poor water solubility [15,16]. The hydrophobic nature of these ESSOs can be overcome by solubilizing them in NE which is a dispersion system that combines the oil with water by the means of surfactants and co-surfactants [17].

NE based on ESSOs of Ch or Gar may potentially enhance anti-neoplastic effects of the incorporated chemotherapeutic candidate on malignant tissues and reduce the cytotoxic effect on healthy cells due to the small size of the NE droplets and natural chemopreventive and chemotherapeutic effect of ESSOs. They may have great applications in drug delivery as they can be administered through various administration routes [18]. To our knowledge, this will be the first attempt to evaluate the *in vitro* efficacy and cytotoxic effects of the novel formulas MMC loaded -NEs based on either Ch or Gar ESSOs on the HeLa cell line.

2. Materials and methods

2.1. Materials

Gar and Ch oils were obtained from Sokar nabat for natural oils (Jeddah, KSA). Tween 80 (T80), span 20 (S20), ethanol, formaldehyde and distilled water were obtained from Al Shafei medical and scientific equipment, Est (Jeddah, KSA). Mitomycin C (Mitonco®) was purchased from Korea United Pharma, Inc. Dulbecco's modified eagle medium (DMEM), fetal calf serum (FCS), trypsin, penicillin streptomycin and dimethyl sulfoxide (DMSO) were obtained from UFC biotechnology, Inc. (Jeddah, KSA). Phosphate buffered saline (PBS) was purchased from Biodiagnostic and research reagents (Cairo, Egypt). Sterile water for injection was gifted from King Abdulaziz University Hospital. Coomassie brilliant blue was procured from biomatik (Ontario, Canada). The 3(4,5dimethylthiazole- 2-yl)-2, 5 diphyneltetrazolium bromide (MTT) assay was purchased from Cayman's chemical (Michigan, US). Trypan blue (0.4%), 4′,6-diamidino-2-phenylindole

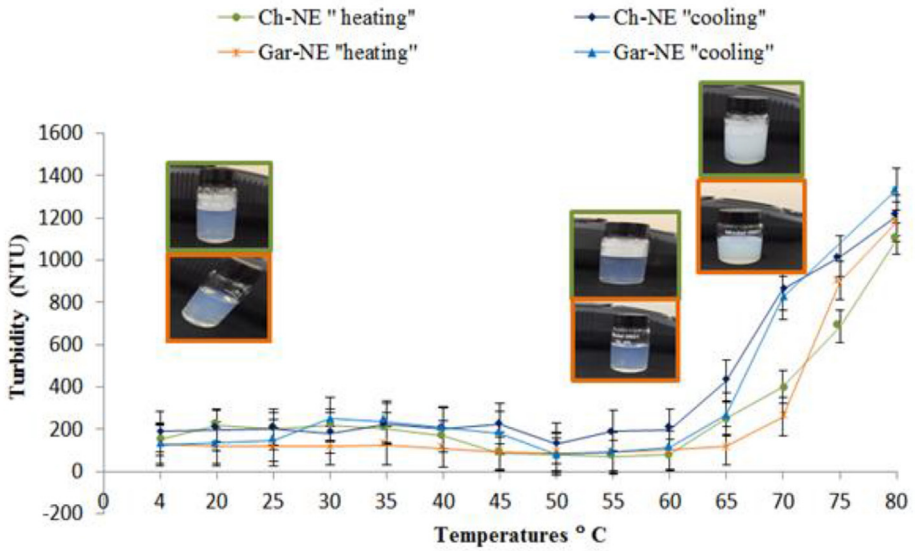

Fig. 2. Turbidity-temperature profile of NE based-ESSO. The images show the changes in turbidity of the formulas at different temperatures.