RESEARCH ARTICLE

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Plant Based Extract Oil-Based Nano emulsions: Impact on Human Melanoma Cell Line

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Abstract

Background: Cancer is a challenge for either the patient or the healthcare manager. Treatment protocols based on chemotherapy or radiotherapy, or both are interfering with the patient's life making him suffer rather than being alleviated. This burden pushed the scientists to search for new regimens that may help ameliorate patient as well as doctor inconvenience. Benefits of plant extracts as medical substitutes in cancer management have been proved. New nano formulated drug delivery systems may help overcoming remedy regimens barriers and obstacles. The present research topic aims to evaluate the anticancer power of two plant extracts in nano emulsion formulation on human melanoma cell line. **Methods:** Carvacrol and rosemary essential oils were obtained, and nano emulsions were formulated. NE were characterized using TEM for charge and size distribution. The A375 human melanoma cell line was cultured and propagated then IC_{50} of prepared NE was added. Assessment of cell cytotoxicity, effect on angiogenesis and apoptosis were tested. **Results:** After synthesis and characterization, both carvacrol nano emulsion (CNE) and rosemary nano emulsion (RNE) were capable of inhibiting melanoma cell line viability, angiogenesis and they enhanced the expression of caspase-3 proapoptotic marker. **Conclusion:** Rosemary and carvacrol extract nano emulsions could be a new revolutionary agent in human melanoma therapy and these formulations can be applied locally.

Keywords: Carvacrol- Rosemary- Nano emulsion- Plant extract- Cell line- Essential Oil

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Introduction

Mortality caused by cancer is still high to our day and it is a serious worldwide problem. This may be caused by the late discovery of the disease as well as the low socioeconomic levels preventing or limiting further treatment. One of these discovered malignancies is melanomas. Melanomas are skin cancer representing the sixth most commonly occurring type of cancer around the world. Its incidence has increased recently due to damaging rays surrounding us. The national cancer institute said that melanomas deaths are more than 80% of deaths caused by cancer [1]. Melanoma is a rapidly progressing, life-threatening condition with a very poor prognosis. Treatment protocols are radiotherapy, chemotherapy, surgery, or combinations. Antineoplastic drugs worsen the patient quality of life as they have many side effects. Surgical removal of tumors is not always amenable due to its late diagnosis in advanced stages in which metastasis has already occurred. Radiotherapy to melanomas of the head and neck region destroys vital structures in the tumor field. All of the above lead to failure of treatment regimens. This failure is also related to resistance of melanoma cells to chemotherapy lowering the melanoma patient survival rate below five years. This is why scientists are trying to combine many anti-melanoma drugs to overcome this resistance [2].

Recently, clinicians are trying new technologies to deliver anticancer drugs to minimize their doses. The emergence of nanotechnology revealed a new family of drug delivery systems [3]. Nanoscience is in focus in pharmacological sciences. It is, in other words, the formulation of drugs in a nanometer range. This very small size helps and allows drugs to better penetrate target tissues and to come in close contact so that they can deliver active drug components more efficiently to diseased areas. This newly applied form of drugs has lower toxicity and drug side effects. It also reduces drug intake frequency and dosage so enhances patient satisfaction [3].

Nanometric drug carriers have various forms as nanoliposomes, nanodrugs, nano capsules as well as nano emulsions [4]. Carriers in this small range of size ensure delivery of active ingredients of drugs to target tissues and minimize drug toxicity to the least [4]. Plant based essential oils (EO) have wide and diverse bioactivities. They consist mainly of terpenes with variable chemical structures. Their anticancer properties have been widely studied and proved. They have cancer cell targeting power either used solely or combined with known chemotherapeutic agents such as paclitaxel and

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5-fluorouracil [5,6].

Rosemary and carvacrol essential oils are examples of these plant-based polyphenols. They exert diverse pharmacological effects. Both have been studied for their anticancer power [7]. RE proved to exert an antitumoral effect against malignant cells of colonic cancer in many studies. This was conducted by inhibition of cell growth as well as cell cycle arrest and induction of apoptosis. RE also enhanced the anticancer impact of 5-fluorouracil on resistant cells [8]. The Carvacrol counter cancer effect can't be denied based on many previous studies. It upgrades the oxidative stress in mitochondria so guides the cells towards apoptosis. Its role in inhibiting tumor progression is well known [7].

Carvacrol (CV, 5-isopropyl-2-methylphenol) is a monoterpene extracted from several plants such as thymus vulgaris, citrus aurantium and Origanum vulgare. Although its biological potentialities have been proved, its partial solubility in water and its instability facing light, heat and pressure limits its use commercially [8,9].

Rosemary, being one of the mint family members, has attracted scientists' attention for its potent antitumor impact. It puts brakes on cancer cell growth. As one of the essential oils, it isn't fully soluble in water and has ethanol or acetone as solvents [10,11]. One of the nanoscience emergencies today is nano emulsions, especially oil in water(O/W) trying to overcome the EO poor solubility in water. In this nano phase, the active EO is dissolved in the inner phase of the emulsion [12]. This form helps overcome the EO insolubility and allows it to penetrate inside cells to exert its beneficial effect. To study a new drug or even a new delivery method, researchers start at the cellular level then animal models are developed followed by its application on human volunteers. Cell lines undergo similar genetic changes found in tumors. This may explain why in-vitro assessment is of great importance in new drug evaluation [13]. The goal of this work is to test and compare RNE and CNE impact on human melanoma cell line to make a formula in the future that can be locally applied for melanoma of skin and mucous membrane.

Materials and Methods

All experimental protocols were reviewed and approved by the Medical Research Ethical Committee of the National Research Centre (NRC) number (MREC-NRC): 19246. The procedures used in this study adhere to the standards of the Declaration of NRC.

Carvacrol and Rosemary Nano-Emulsion Preparation

Carvacrol and rosemary essential oils were obtained from the Cell Culture Department - Nawah Scientific - Cairo, Egypt. Oil in water (O/W) nano emulsions were prepared by adding carvacrol and rosemary oil (1.5%), medium chain triglyceride (MCT), lecithin and non-ionic surfactant and emulsifier polysorbate 80 in water under constant shaking conditions. Nano emulsions were prepared in an aqueous medium by adding oils with MCT, lecithin and polysorbate 80 in the weight ratio (1:1:0.01:2) and stirred vigorously at 500 rpm for 5 min. Finally, the whole was sonicated at 20% Amplitude with 4 sec on and 2 sec off cycle in a sonicator (Ultrasonic Processor, GEX 750, Newtown, CT) with 750 W for 10 min. Samples were surrounded by ice to avoid the heat generated from the sonication process.

Characterization of nanoparticles using TEM

Particle size and shape of the CNE as well as RNE was determined with a JEOL JEM 1011 (Japan) transmission electron microscope. A 400 µL of nanoparticle solutions will be deposited on carbon coated copper grids (400 meshes) and dried at 30 °C before image capture.

Particle size distribution and zeta potential identification

The prepared particles were analyzed for their particle size and size distribution in terms of the average volume diameters and polydispersity index by photon correlation spectroscopy using particle size analyzer. Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom) at fixed angle of 173° at 25° C. Samples were analyzed in triplicate. The same equipment was used for the determination of zeta potential. The zeta potential (ζ) will be calculated from the electrophoresis mobility using the Smoluchowski's equation.

$$\zeta = (\eta / \varepsilon) \times \mu e$$

Where, η is the viscosity and ε the permittivity of the medium.

Cell line propagation & maintenance

Human melanoma (A375) cell line was supplied from Cell Culture Department-VACSERA- EGYPT. Cells were imported from the American Type Culture Collection (ATCC) in the form of a frozen vial with the reference number "CRL1619". Cells were homosapiens (Human), confirmed as human by immunofluorescence. Cells were grown in DMEM-medium (4,500 mg/ml of glucose, gluta MAX and sodium pyruvate) supplemented with 10% fetal calf serum (FCS), 50 U/ml penicillin and 50 Ig/ml streptomycin, at 37 °C in a 5% CO2 humidified atmosphere. Media was changed every other day till reaching 70-80% confluence. Then cells were passaged to increase their numbers. Each cell line was then divided into groups. Nano emulsions were added to the experimental plates and kept for 48 hours. All subsequent tests were performed simultaneously.

Assessment of CNE and RNE cytotoxicity on Human melanoma (A375) cell line

Measurement of cell viability and proliferation comprises the underlying basis for numerous in vitro assays directed towards the quantitation of a cell population. The SRB assay has been used since its development in 1990 to inexpensively conduct various screening assays to investigate cytotoxicity in cell-based studies [14]. This method relies on the property of SRB, which binds stoichiometrically to proteins under mild acidic conditions and then can be extracted using basic conditions; thus, the amount of bound dye can be used as a proxy for cell mass, which can then be extrapolated to

measure cell proliferation.

Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5x10^3cells) were added in 96 well plates and incubated incomplete media for 24 hours. Cells were treated with another aliquot of 100 µL media containing CNE. After 48 h of drug exposure, cells were fixed by replacing media with 150µL of 10%TCA and incubated at 4°C for 1h. The TCA solution was removed, and the cells were washed 5times with distilled water. Aliquots of 70µL SRB solution (0.4%w/v) were added and incubated in a dark place at room temperature for 10min. Plates were washed 3times with 1% acetic acid and allowed to air-dry overnight. Then, 150µL of TRIS (10mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540nm using a BMGLABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

The percent cell cytotoxicity was calculated by means of the formula:

% Cytotoxicity = O.D of Control sample- O.D of Test sample/ O.D of Control Sample

Photomicrographs of melanoma cells grown in different concentrations of both nano emulsion preparations were taken using inverted light microscope as well as for control cells. (Olympus, Tokyo, Japan).

IC 50 calculation for CV and RE nano emulsions.

Evaluation of antiangiogenic effect of CV and RE nano emulsions on A375 cell line using ELISA measuring for VEGF and Statistical analysis:

Assay Principle

In principle: the ELISA plate is pre-coated with an antibody specific to human VEGF-A. After adding samples/standards, a biotinylated detection antibody specific for human VEGF-A and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each well and incubated washing and the substrate is added after around 15 min stop the solution is added to stop the reaction. The optical density of VEGF-A conjugated with the biotinylated detection antibody is measured. Concentrations of VEGF-A in cell culture media will be measured with ELISA.

Statistical analysis

Statistical analysis was performed with SPSS 16 ® (Statistical Package for Scientific Studies), Graph pad prism & windows excel and presented in 2 Tables (Table 1, Table 2) and 2 graphs. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that data originated from normal data. Accordingly, comparison between 3 different groups was performed by One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparison.

Evaluation of apoptotic markers in A375 cell line following treatment with CNE and RNE nano-emulsions using western blot

Antibodies to caspases-3, Bcl-XL, BAX was purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-GADPH was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP conjugated secondary antibodies was purchased from Amersham Pharmacia Life Sciences (Rockford, IL, USA). Standard western blot analysis was carried out as previously described.

Results

Transmission electron microscopy (TEM)/ Particle size and zeta potential identification

The prepared nano emulsion is mainly spherical in shape with particle size varying between 220.5±3.195 nm. The particles are separated from each other which reflects the capping action of the nano emulsion in the preparation process. The mean droplet size diameter of CNE was 150 nm (25% ≤ 50 nm, while 75% ≤ 150 nm. The droplets of RNE have a size ranging from 20 to 224 nm. CNE is stable with the zeta potential ranges of -15.1±0.907. RNE is stable with the zeta potential ranges of -8 to -10 mV (Figure 1).

Cytotoxicity/IC_{50} calculation and cell morphology assessment

Both nano emulsion preparations of carvacrol and

Table 1. Descriptive Results of All Groups

	N	Minimum	Maximum	Mean	Std. Deviation	P value
RNE + A375 cell line	10	198.19	287.16	231.73	31.32	<0.0001*
CNE + A375 cell line	10	214.78	333.89	286.18	42.88	
A375 Control Cell line	10	366.36	485.12	417.72	43.09	

**, Highly significant difference as P<0.001.

Table 2. Tukey's Post Hoc Test for Multiple Comparisons

Test details	Mean 1	Mean 2	Mean difference	Standard error of difference	95% Confidence interval	P Value
RNE + A375 vs. CNE + A375	231.7	286.2	-54.45	17.66	-98.23 to -10.67	0.0126*
RNE + A375 vs. A375 Control	231.7	417.7	-186	17.66	-229.8 to -142.2	<0.0001**
CNE + A375 vs. A375 Control	286.2	417.7	-131.5	17.66	-175.3 to -87.76	<0.0001**

*, significant difference as P<0.05; **, Highly significant difference as P<0.001

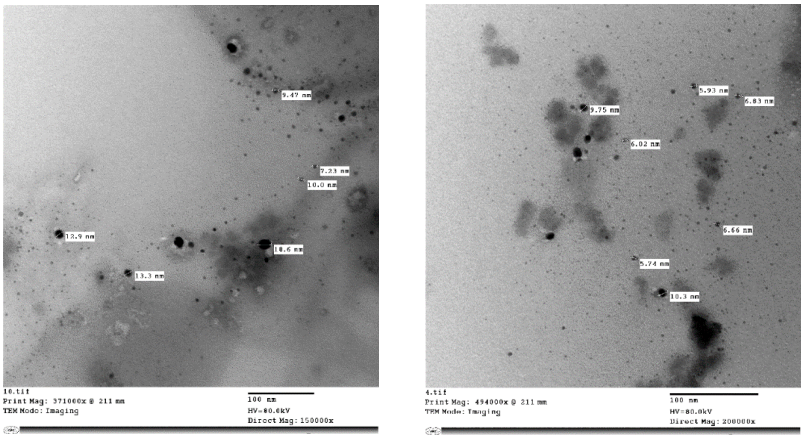

Figure 1. TEM Image of CNE & RNE [Scale =100nm]. RNE particle size varying between 220.5±3.195 nm while CNE mean droplet size was 150 nm (25% ≤ 50 nm, while 75% ≤ 150 nm.

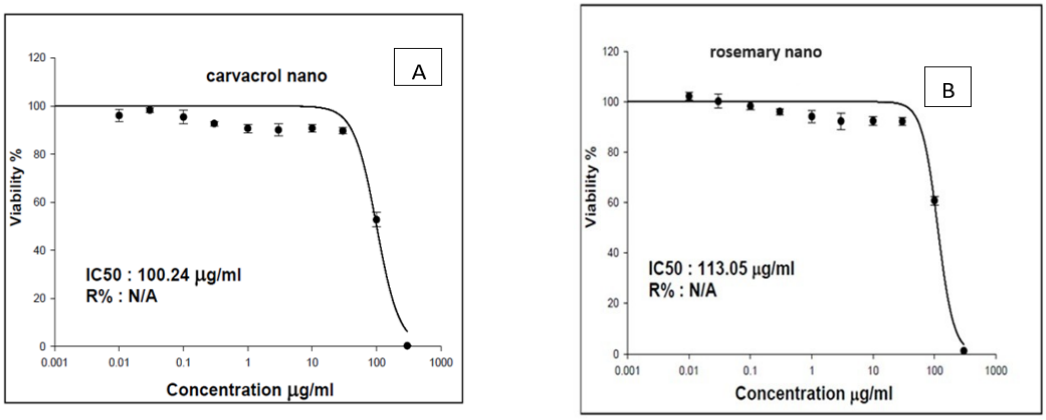

Figure 2. Cytotoxic Potential Nanoemulsions against A375 Cell Line; CNE IC 50 = 100.24 µg/ml (A) and RNE; IC 50 = 113.05 µg/ml (B).

rosemary were cytotoxic for melanoma cells in a dose dependent manner with an IC_{50} of 100.24 µg/ml and 113.05 µg/ml respectively (Figure 2). Photomicrographs showed decreased viability and proliferation with many floating dead cells. This effect was pronounced with a dose of 300 µg/ml (Figure 3,4).

Effect of CNE and REN on VEGF expression in A375 cell line using ELISA

Statistical analysis was performed with SPSS 16 ® (Statistical Package for Scientific Studies), Graph pad prism & windows excel and presented in 2 Tables and 2 graphs. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that data originated from normal data. Accordingly, comparison between 3 different groups was performed by One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparison. Comparison between all groups revealed highly statistically significant difference as P<0.0001. Multiple comparisons revealed that RNE + A375 cell line was significantly the lowest (231.73 ± 31.32), then CNE

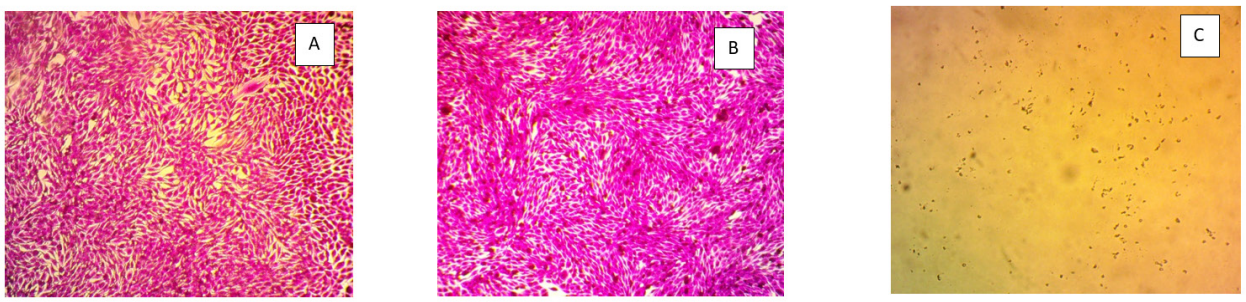

Figure 3. Inverted Microscope Photomicrograph for A375 Cell Line Cultured in CNE with Various Conentrations (A:control, B:30 µg/ml, C: 300 µg/ml). Photomicrographs showed decreased viability and proliferation with many floating dead cells. This effect was pronounced with a dose of 300 µg/ml.

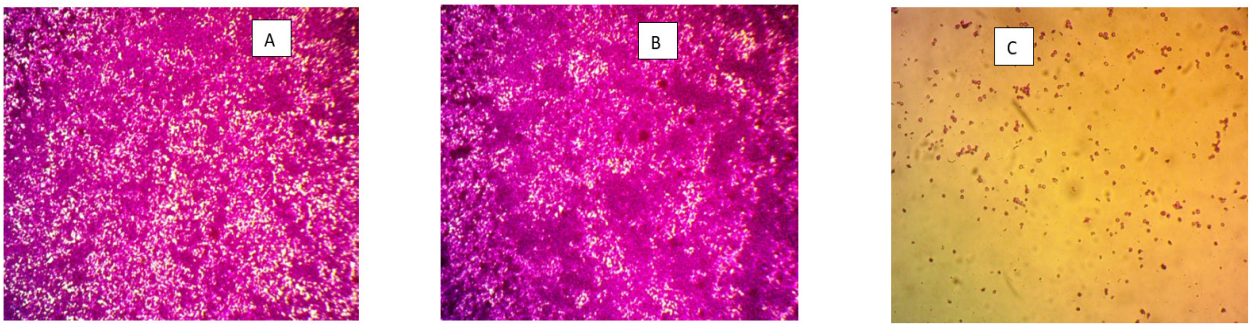

Figure 4. Inverted Microscope Photomicrograph for A375 Cell Line Cultured in RNE with Various Conentrations (A:control, B:30 µg/ml, C: 300 µg/ml). Photomicrographs showed decreased viability and proliferation with many floating dead cells. This effect was pronounced with a dose of 300 µg/ml.

+ A375 cell line (286.18 ± 42.88), while A375 Control Cell line (417.72 ± 43.09) was significantly the highest.

Impact of CNE and RNE on apoptotic profile of melanoma cells

Compared to control untreated melanoma cells, CNE in its IC50 could express caspase-3 proapoptotic protein and inhibit Bcl-XL antiapoptotic protein. RNE in its IC50 could express caspase-3 proapoptotic protein while Bcl-XL antiapoptotic protein couldn't be detected in all cells (Figure 5).

Discussion

Around the world, cancer has a pivotal role in raising the rate of mortality. This isn't only due to cancer pathogenesis, but also due to treatment protocols having life threatening side effects. Clinicians till today prescribe these cancer killing agents as the first line medications [15]. This may explain why researchers are in a hurry

Figure 5. (A) Western blot images for apoptotic profile markers in A375 cell pellets cultured for 48 hours in CNE 113.05 µg/ml. (B) Western blot images for apoptotic profile markers in (A375) cell pellets cultured for 48 hours in RNE 100.24 µg/ml.

to find alternatives. The Plant kingdom has been widely studied as they possess many health benefits with negligeable side effects [16].

Rosemary and Carvacrol are descendants of this family with a remarkable anti-tumor impact. Extract from rosemary proved to exert antioxidant, anti-inflammatory, as well as anticancer properties. Carvacrol can be obtained from a variety of plants such as oregano and thyme. Its anti-proliferative effect has been studied against colon and lung carcinoma as well as neuroblastoma. Unfortunately, its instability and low solubility in water are obstacles limiting its medical use [17,18]. Nanotechnology is an emerging field of bioscience. It deals with structures in a nanometer range of size. Their large surface area compared to volume distinguishes them from larger size structures. This opens a new era of use especially in medicine and drug delivery [2].

Smart drug delivery systems have been possible after nanotechnology application especially as vehicles carrying diagnostic as well as therapeutic agents for tumor management. Examples of these vehicles are nano shells, dendrimers, and nano emulsions [19]. Nano emulsions have the benefits of increasing drug efficiency while lowering their toxicity and maintaining a constant therapeutic level of the drug used. NE allows the use of new entity of agents that were known to be insoluble in water. This limited their use before. But now, we can gain benefits from their use [20].

Choudhoury et al. presented an NE preparation of paclitaxel in 2014. He proved a better bioavailability as well as a sustained release compared to its IV form. When applied on a mouse model, these two parameters improved by 55.9% [20]. Melanoma cell line was studied by others as it is an aggressive skin and mucous membrane malignancy with a lower survival rate and a bad prognosis when diagnosed. Kretexer et al presented paclitaxel lipid NE capable of binding LDL(Low Density Lipoprotein) receptors. This helped decrease drug toxicity and enhanced anticancerous impact. Combining simvastatin with paclitaxel NE in a melanoma mouse model improved these NE antitumoral effects by increasing LDL receptors expression so enhancing NE internalization. Cholesterol NE succeeded in decreasing tumor size of murine melanoma model by 50% and resulted in 10% cytotoxicity of melanoma cell line [21].

NE are excellent for delivering drugs to skin and dermal sites as mucosal surfaces. So, they are typical and ideal for topical formulations as in case of infection, psoriasis, and malignancy of skin [21]. The combination of oil, water and detergents will help improve drug insolubility, instability, and bioavailability. They are absorbed by the rich vascular plexus surrounding cancer cells where they accumulate to exert their effect [22].

Cancer research focuses on NE because they have characteristics that help ameliorate their power to fight against cancer. Added to their nanometric size, they are superficially charged and have target cell specificity [23].

In this research, synthesis of NE from carvacrol and rosemary was carried out and characterization of these formulations was done by electron microscopy as well as dynamic light scattering. RNE and CNE showed a size in the nanometric range (CNE 220.5±3.195 nm) (RNE 20 to 224 nm) with a surface charge negative enough to allow stability (CNE -15.1±0.907) (RNE -8 to -10 mV). This is successfully in accordance to previously published results explaining that the highly negative surface charge stabilizes the emulsion by inducing an electrostatic repulsion [19].

Scientists are incorporating plant-based extracts in these NE to strengthen their stability and ameliorate skin and mucous membrane permeability while the releasing effect is sustained [24]. Monge-Fuentes et al loaded NE with acai oil and tested them as photosensitizer in vitro for NIH/3T3 normal cells and B16F10 melanoma cell line. Results were promising by death of 85% of malignant cells and reservation of normal cells viability. In mice model of melanoma, these NE decreased tumor volume by 82% [25]. In the present study, RNE and CNE inhibited melanoma cells successfully in a dose dependent manner. IC 50 of CNE was lower than RNE showing better antiproliferative ability (CNE IC50 = 100.24 μg/ml and RNE; IC50 = 113.05 μg/ml).

In accordance, Eid et al assessed the antiproliferative potential of R. Officinalis nano emulgel against different Hep 3B and Hela cancer cell lines and showed a potent antiproliferative activity [26]. Similarly, El otaibi 2021 showed that RE-NE enhanced mitomycin C effect on Hela cells and MCF-7 cells by decreasing viability and IC50 as well as inducing apoptosis even at low doses of MC [27].

The ability of malignant cells to replicate and increase the tumor size depends largely on their ability to form new blood vessels. So, drugs targeting the angiogenesis process are effective in limiting tumor volume. Till now, the exact role of VEGF may not be fully revealed but its role in endothelial cell proliferation and in the induction of proteins antagonizing apoptosis so preventing endothelial cell death can't be neglected [28,29]. In the presented work, RNE as well as CNE were able to lower VEGF expression by melanoma cell line with RNE impact being the most effective 231.73 ± 31.32 while CNE 286.18 ± 42.88 compared to control untreated melanoma cells.

Similarly, El-Oteibi et al showed that rosemary nano emulgel induced a synergism with MC and enhanced apoptosis of MCF-7 and Hela cells [27]. Also, Carvacrol can only induce a proapoptotic effect on malignant cells in high concentrations (0–100 μM/L) [30]. Khan and his colleagues proved that ROS induction in mitochondria of A549 cells enhanced apoptosis and this is responsible for its anticancer effect [31]. In a study the impact of CV/ chitosan nanoparticles has been assessed. It showed promising anticancer effect in breast and cervical cancer cell lines. They combined CV nanoparticles with doxorubicin and topoisomerase inhibitors. It proved to exert a promising effect in cancer treatment, as it ameliorates the stability of CV with targeted delivery and less resistance to dose [32].

Innovation in technology and advancing forms of drugs will help bypassing physiochemical burdens of drugs. This may be in the form of natural bioactive compounds or EO in nanoforms of natural origin [33].

In line with the results of the present study, Rosemary

and Zataria were assessed nano liposomal form. Their cytotoxicity in HepG2 cells were higher than the EO themselves. Nanoliposomes enhanced apoptosis and stopped cell cycle in G2 phase indicating their antiproliferative effect [34]. In another study, RE nanoparticles were induced cytotoxicity to Hep-2 cells in a dose-dependent manner. This was supported by the induction of apoptotic changes and cell cycle arrest at G2/M phase. They also could enhance ROS expression in malignant cells. Autophagosomes were detected in rosemary nanoparticles treated cells [35]. Proving the benefits of EO NE cancer delivery systems, Heracleum persicum EO NE exhibited anticancerous potentials both in vitro and in vivo models of breast cancer. H. persicum EO NE showed a nanotherapeutic alternative to well-known chemotherapeutic agents. Added to its cytotoxicity to malignant cells and its antioxidant properties, it arrested cell cycle and enhanced apoptosis [36].

EO showed a synergistic effect when combined with chemotherapeutic agents. This could help reduce the given dose of the drug so minimizing its undesirable side effects. Kaur et al studied the efficacy of a combination of paclitaxel and erucin in reducing breast cancer cell resistance. This frankincense oil-based NE showed a promising line of managing malignancy of breast with resisting cells. Also, this combination helped restoring malignancy-damaged breast tissue to normal one [37].

NE formulations helped scientists to overcome the instability of EO. Manaa et al used this formulation to enhance the stability of oregano EO so that we can get benefit of its biological properties. The NE form significantly decreased the IC 50 of the free form and the conventional emulsion and upregulated the pro-apoptotic markers. In a similar way, NE of oregano inhibited colony formation of two cancer cell lines of human adenocarcinomas [38]. The EO instability facing environmental conditions is a major burden for clinicians. NE favors and enhances stability of EO to stress and heat. In another study, NE of EO was chosen to bypass the poor solubility that limited the clinical use. Mentha arvensis EO in NE oil-based formulation was evaluated as a promising anticancer agent. It induced early apoptotic changes in aggressive cell line of thyroid carcinoma proved by annexin-v FITC. MTT showed that NE were cytotoxic to malignant cells in a dose lower than the free form of EO. The nanometric range of particles allows for a better penetration of blood vessels enhancing the EO effect [39].

Optimization of NE before commercialization is a crucial step for safety evaluation and for ensuring optimal delivery of active ingredients in right doses [40]. It must be taken into consideration that toxicity from these nano sized drugs wouldn't be easily detected in the human body. From the presented data, RE and CV nano emulsions showed antitumoral effect against cultured human melanoma cell line. They were cytotoxic as they inhibited cell growth and enhanced their pro-apoptotic profile. They downregulated angiogenic marker VEGF. So, they may have promising anticancer therapeutic effect especially if used locally as the nano emulsions have greater skin and mucous membrane penetration. If used locally, this will be very beneficial in melanomas treatment as they are skin and mucous membrane malignancies. This will allow decreasing the need for systemic chemotherapy or deleterious radiotherapy. As they are phytocompounds, their side effects are expected to be minimal. But further trials taking a long way on animal models are needed before starting clinical trials and before setting these agents as anticancer drugs.

In Conclusion, rosemary and carvacrol extract nano emulsions could be a new revolutionary agent in melanoma therapy if presented in local formulations. Conventional drug delivery systems lack stability and high internalization power offered by NE. NE steadiness makes them suitable and ideal for local application. NE uses in the medical field, especially oncology, opens new frontiers to escape side effects of chemotherapy. Incorporating herbal extracts in these NE will help by passing current obstacles in cancer therapy.

Author Contribution Statement

Dr/ Marwa M. Ellithy is the corresponding author. She made the study design. She, helped by Dr/ Reham, wrote the literature review. They also fulfilled the experimental procedures. Statistical analysis was conducted by Dr/ Reham Ahmed. All authors listed have significantly contributed to manuscript preparation, writing and editing.

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Recommendations

Further studies comparing the effect of CV and RE nano-emulsions to already known chemotherapeutic agents are needed. Researchers should use animal models of melanoma prior to preclinical studies on human volunteers to evaluate the exact dose and time of treatment. Plasma levels of these active components must be assessed after being locally applied on skin or mucosa. This will reveal any undesirable side effects of these local formulations.

Ethics approval and consent to participate.

Declarations Ethics approval and consent to participate Approval was obtained from the Medical Research Ethics Committee of National Research Centre Number (MREC-NRC): 19246. The procedures used in this study adhere to the standards of the Declaration of NRC.

Data availability statement

Data included in article/supplementary material/ referenced in article.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

List of abbreviations

CV: Carvacrol
NRC: National Research Centre
O/W: Oil in water
CNE: Carvacrol nano emulsion
RNE: Rosemary nano emulsion
MERC-NRC: Medical Research Ethical Committee of the National Research Centre
EO: Essential Oil
MCT: medium chain triglyceride
SPSS: Statistical Package for Scientific Studies
LDL: Low Density Lipoprotein
NE: Nano emulsion
DLS: Dynamic Light Scattering
OD: Optical Density
TEM: Transmission Electron microscope
SRB: Sulforhodamine
ANOVA: One way Analysis of variance
AHP: Avidin-Horseradish Peroxidase
IC50: minimum inhibitory concentration
H: Hour

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