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Preparation of a nanoemulsion containing active ingredients of cannabis extract and its application for glioblastoma: in vitro and in vivo studies

Houra Mobaleghol Eslam¹, Fatemeh Hataminia¹, Fariba Esmaeili¹, Seyed Alireza Salami^{2,5}, Hossein Ghanbari^{1,3} and Amir Amani^{4*}

Abstract

Recently, the anti-tumor effects of cannabis extract on various cancers have attracted the attention of researchers. Here, we report a nanoemulsion (NE) composition designed to enhance the delivery of two active components in cannabis extracts (Δ^9 -Tetrahydrocannabinol (THC) and Cannabidiol (CBD)) in an animal model of glioblastoma. The efficacy of the NE containing the two drugs (NED) was compared with the bulk drugs and carrier (NE without the drugs) using the C6 tumor model in rats. Hemocompatibility factors (RBC, MCV, MCH, MCHC, RDW, PPP, PT and PTT) were studied to determine the potential in vivo toxicity of NED. The optimized NED with mean \pm SD diameter 29 ± 6 nm was obtained. It was shown that by administering the drugs in the form of NED, the hemocompatibility increased. Cytotoxicity studies indicated that the NE without the active components (i.e. mixture of surfactants and oil) was the most cytotoxic group, while the bulk group had no toxicity. From the in vivo MRI and survival studies, the NED group had maximum efficacy (with ~ 4 times smaller tumor volume on day 7 of treatment, compared with the control. Also, survival time of the control, bulk drug, NE and NED were 9, 4, 12.5 and 51 days, respectively) with no important adverse effects. In conclusion, the NE containing cannabis extract could be introduced as an effective treatment in reducing brain glioblastoma tumor progression.

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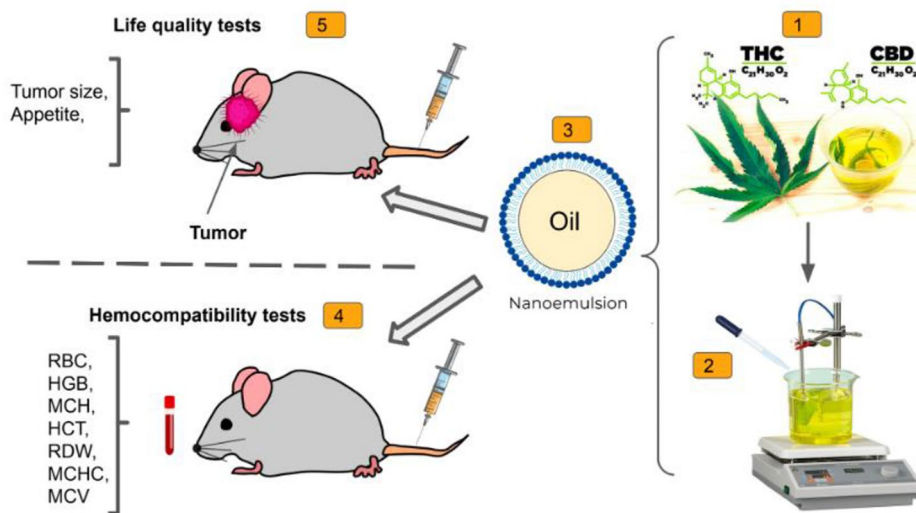
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Graphical abstract



Keywords Glioblastoma, In vivo model, Nanoemulsion, THC, CBD, Hemocompatibility

Introduction

Glioblastoma multiforme is a malignant brain tumor which is known as extremely aggressive with generally poor prognosis for patients. Treatment depends on the stage of the disease and includes radiotherapy, chemotherapy and surgery. However, problems with hidden cancerous cells remain important challenges [1, 2]. The two central challenges in the treatment of glioblastoma (GB) are the inability of many antineoplastic drugs to cross the blood-brain barrier (BBB) and their noticeable adverse effects. The efficacy of anticancer medicines is currently unsatisfactory, primarily due to delivery obstacles, particularly those caused by the blood brain barrier [3].

In recent years, natural products with therapeutic potentials have attracted increasing attention. An example of a plant with medicinal potential is *Cannabis sativa* L., which has been used for thousands of years [4]. The primary active component of cannabis, Δ^9 -tetrahydrocannabinol (Δ^9 -THC or THC), as well as the non-psychoactive cannabidiol (CBD), have been extensively studied [5]. Several effects have been reported from CBD and THC, including elongation of sleep, alleviation of neuropathic pain, stimulation of appetite, anti-inflammatory effects and antiseizure properties [6]. The combined administration of THC and CBD for the treatment of various diseases is being examined for its potential therapeutic value [7–9]. THC and CBD inhibit the proliferation and survival of cancer cells [7]. Therefore, the combined use of both agents—as employed in our study—is frequently observed in the literature.

Nanomedicine can provide a safe and effective method to deliver herbal compounds. Studies have shown that delivery systems based on nanomedicine, such as nanoemulsions (NEs), nanocapsules, phytosomes, and nanoliposomes, can improve the half-life, bioavailability, and pharmacological activity of plant compounds [10]. NEs are colloidal dispersions of two immiscible liquids which have been stabilized using emulsifiers and form a single-phase system [11, 12]. Many studies have been performed on NEs as drug delivery systems [13]. Using a self-emulsification method with cannabis extract, Dvora Izgelov et al. generated NE with nano-sized droplets (<50 nm). The formulation was administered orally to an animal model. The pharmacokinetic parameters, such as C_{max} and AUC, increased up to 2-fold in the nanoformulation. Interestingly, both the taste of the oil and the gastrointestinal absorption of the drug improved [14]. Furthermore, CBD was formulated into lipid nanocapsules (LNCs) with sizes of 20 nm and 50 nm. It was shown that the smaller LNC reduced the IC_{50} against the human glioma cell line U373MG up to 3 fold [15]. The effects of CBD coated with nano-chitosan on memory and learning were investigated in a rat model of Alzheimer's disease. Compared to those in the control group, the delay in response of the nano-chitosan-coated CBD group was significantly shorter, and the expression of the CB1 and CB2 proteins increased significantly. Nano-chitosan-coated CBD appeared to have the potential to reduce beta-amyloid plaques, increase CB1 and CB2 levels, and improve memory and learning in the rat animal models [16].

In this study, we prepared a nanoemulsion of hemp seed oil containing THC and CBD (NED) to evaluate its efficacy in an animal model of glioblastoma. Various tests, including cytotoxicity assays using C6 glioma cell lines, hemocompatibility assessments, as well as in vivo efficacy studies in Wistar rats, were employed. The objective of the study was to investigate the potential advantages of the nanoformulation in treating glioblastoma, assessing factors such as cell viability, blood compatibility and tumor growth.

Materials and methods

Materials

Mixture of THC and CBD was obtained from the Industrial and Medical Cannabis Research Institute (IMCRI), Iran. Tween 80 and Span 80 were purchased from Merck Chemicals (Germany). The water was deionized by Human Corporation Filter (Korea). DMEM (Dulbecco's Modified Eagle Medium), trypsin/EDTA, F12, penicillin and streptomycin were purchased from Gibco (USA).

Preparation of NED

The NED was prepared using the titration method. A mixture of THC and CBD (1:1) at a final concentration of 300 ng/mL was infused to hemp seed oil (1.00 w/w%). The mixture was then filtered to obtain a clear oily phase. Span 80 (7.82% w/w) was added to the oil phase. The mixture was stirred at 4000 rpm for 10 min. Then, 27.18% w/w Tween 80 and 10% ethanol were added to the mixture in the same conditions. Subsequently, deionized water was added dropwise to the container.

Construction of pseudo-ternary phase diagram

Surfactants and co-surfactant (Smix) were combined in constant weight ratios of 1:1, 3.5:1, and 7:1. Samples that remained transparent with no sign of phase separation were considered as stable in the pseudo-ternary phase diagram.

Accelerated stability studies

Different accelerated stability tests, including centrifugation (5000 rpm for 30 min), heating-cooling cycles (4 °C and 40 °C for 6 times) and freeze and thaw cycles (-21 °C and room temperature for 3 times) were employed to estimate the stability of the prepared samples. The formulations that showed no evidence of instability (i.e. phase separation) were considered as having appropriate stability.

Characterization of the optimized NED

Scatterscope I (K-ONE NANO, Korea) was used to examine the mean particle size of the NEDs. The prepared NEDs were diluted 15 times with deionized water before particle sizing. The data obtained were the particles'

hydrodynamic diameter, (i.e. d_{50}). Also, SPAN was calculated using Eq. (1).

$$\text{SPAN} = (d_{90} - d_{10})/d_{50} \quad (1)$$

The smaller the SPAN is, the more mono-dispersed preparation has been obtained. Transmission electron microscopy (TEM, LEO 906, Zeiss, Germany) was used to investigate the morphology and size of NED. The NED had been stained on a copper grid which was covered with carbon (200 mesh) and placed with 1% of phosphoric tungstic acid at room temperature for five minutes, then, checked by TEM.

The viscosity of NED was determined without dilution with an MCR300 rheometer device (Anton paar, Austria) with concentric cylinders at 25 °C.

Cytotoxicity assay

To determine the cytotoxicity, C6 cell lines (rat glioma cell line) were cultured in DMEM-F12 medium having FBS (10%) and Penicillin/Streptomycin (1%). To evaluate the cytocompatibility, C6 cells were counted and 5000 cells were seeded to each well of 96-well plates and incubated (37 °C, 24 h). Subsequently, 100 μ L of the NEDs were added to each well. After 24 h, 10 μ L of Alamar Blue solution was added to each well and incubated for 4 h at 37 °C. Cell viability was determined by recording the absorbance at 570 and 630 nm using an ELISA microplate reader (BioTek, USA).

Animal studies

All animal experiments followed the guidelines of the Research Ethics Committee and were approved by the Animal Welfare Ethics Committee of North Khorasan University of Medical Sciences (IR.NKUMS.REC.1400.1122). The Wistar rats (male, 10 weeks old, weight ~ 200 g) were purchased from Royan Institute (Iran). Animals were anesthetized by IP injection of ketamine (80 mL/kg/h) and xylazine (10 mL/kg/h) before the animal studies (including cell implantation and magnetic resonance imaging (MRI)). For euthanasia, according to AVMA protocol, the animals were placed inside a CO₂ chamber and CO₂ was gradually introduced. To prevent distress, the CO₂ flow rate was set at 20–30% of the chamber volume per minute. Heartbeat and respiration of the animals were then checked.

Hemocompatibility

To examine the potential in vivo toxicity, male rats' blood was collected from each treatment group. Treatment groups included control (normal saline), bulk (free form of mixture of THC and CBD, 21 μ g per injection), carrier (NE without THC and CBD, 0.7 mL), and NED (NE containing the mixture of THC and CBD drugs, 0.7

mL containing 21 μg of the mixture of THC and CBD per injection). 1 mL of the whole blood was drawn and placed in vacutainers containing EDTA, then, centrifuged (1500–2000 g, room temp, 10–15 min) to separate plasma (top layer), RBCs (red blood cells, bottom layer) and white blood cells. The following hemocompatibility factors were then checked: RBCs, hemoglobin, hematocrit, mean corpuscular volume of RBCs (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW) [17]. For determination of coagulation factors, blood was drawn in a sodium-citrate vacutainer, then centrifuged (3000 rpm, 8 $^{\circ}\text{C}$, 20 min) to obtain platelet-poor plasma (PPP). Prothrombin time (PT) and partial thromboplastin time (PTT) were also evaluated in all groups.

In vivo efficacy studies

Before tumor implantation, rats were carefully positioned in a stereotactic machine. After scalp preparation, a bore-hole was drilled into the skull to inject 5 μL cell suspension containing 800,000 C6 cells at pH 7.4 unilaterally into the right striatum with a Hamilton glass syringe. The coordinates of injection were 1.5 mm lateral from the bregma, 2.5 mm deep from the skull surface and 2 mm posterior. After 15 min of injection, the needle was gently withdrawn, the hole was covered and the incision was sutured. The animals were monitored daily for 14 days post-implantation of the C6 tumor cells.

MR imaging was done in the National Brain Mapping Laboratory (Tehran, Iran) using a 3-T MRI (Slew rate: 200 mT/m/ms, maximum amplitude: 45 mT/m, Siemens Healthcare, Germany). MRI was used to determine the presence, expansion, and tumor size that had been implanted in the rat brain. T2-weighted MR images were

then acquired at different time points. Tumor volume was calculated by drawing areas of interest in each slice at specified time.

Rats with cerebral glioblastoma were divided into 4 treatment groups (5 rats in each group) and treated twice a week via the tail vein. Rat deaths were documented and for each group, the Kaplan-Meier survival curve (generated using Graphpad Prism software) were plotted.

Statistical analyses

The mean and standard deviation were used for all numerical data. Differences between the two-groups and multiple-groups analyses were compared using Student's t-test and one-way ANOVA tests, respectively. For survival analysis, the log-rank test was used. Statistics were considered significant if the p-value was less than 0.05.

Results

Pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were used to study the relationship between the composition of the mixture and its phase behavior. Phase diagrams were generated with Smix values of 1:1, 3.5:1 and 7:1 (see Fig. 1A). The largest amount of oil that could be dispersed in the NE, was 4%, using 45% of the Smix (3.5:1).

Characterization of the optimized NED

From the TEM image, the NED droplets were almost monodispersed in size, having particle size of about 29 ± 6 nm (see Fig. 1B). From DLS results, d50 of NED was determined as 13.1 nm.

The zeta potential of the optimized NED was close to zero (-0.1 ± 0.1 mV) as a result of using the non-ionic surfactants. The viscosity of NED was less than 0.338 (Pa.s). Microscopic examination revealed the existence of

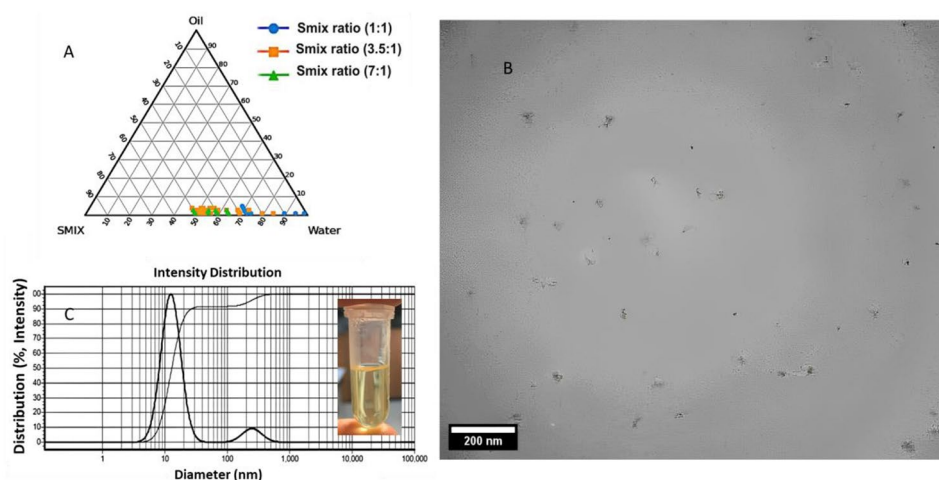


Fig. 1 (A) Pseudo-ternary phase diagrams showing nanoemulsions containing THC and CBD (NED) with different Smix ratios (Blue (1:1), Orange (3.5:1), and Green (7:1)). (B) Particle size and morphology of NED by Transmission electron microscopy (TEM) microscopy, (C) Dynamic light scattering (DLS) results for the NED

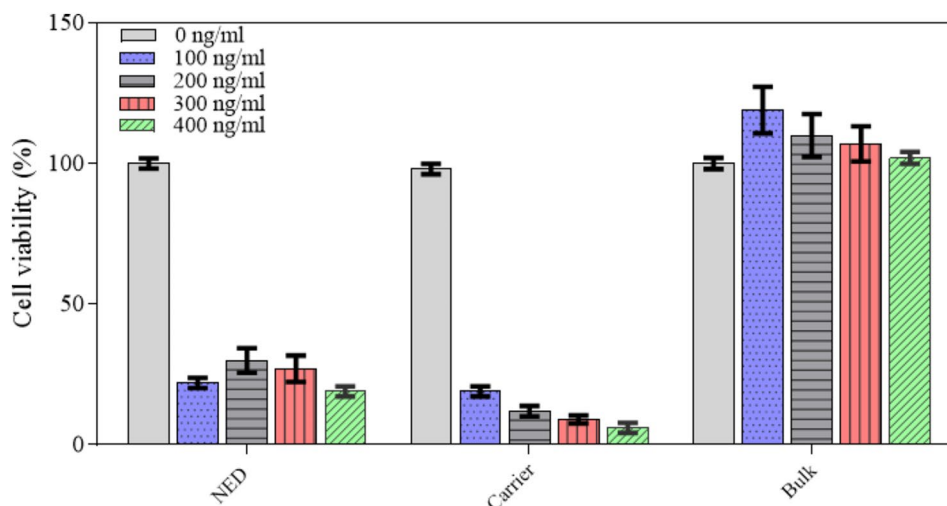


Fig. 2 The results of Alamar Blue assay to determine the C6 cell viability of NED (nanoemulsion containing CBD and THC) and bulk (mixture of CBD and THC) at different amounts of CBD and THC mixture (0, 100, 200, 300 and 400 ng/ml) as well as carrier (nanoemulsion without the drugs) having the same volume to that of NED (the data points of cell viability were presented as mean \pm SD)

spherical droplets. The pH of the NED was determined as ~ 7 (i.e. physiological pH). The NED stability was evaluated over 24 months (Fig. S3) with no important variation in size or transparency.

Cell toxicity assay

In vitro evaluation of the NED toxicity after 24 h was performed using C6 glioma cells and the findings are illustrated in Fig. 2. From the details, increasing the concentration of the drugs in the preparation (CBD and THC) decreased cell viability. The highest cytotoxicity was upon treatment with the carrier group, while the bulk group showed no toxicity and even increased cell growth.

Animal studies

Hemocompatibility

Figure 3 shows the results of hemocompatibility tests for the studied groups. As the details show, the RBC count as well as partial thromboplastin time (PTT) in the bulk group were minimum, followed by those in the NED group. Also, the capacity of RBCs to carry oxygen (HGB), the total volume of oxygen (HCT) and the concentration of hemoglobin in RBCs (MCHC) in the bulk group decreased significantly, compared with the control group. Furthermore, in comparison with the control group, the quantity of hemoglobin (MCH) and variation in size of RBCs (RDW) was significantly higher in the bulk group and NED group, respectively. The remaining parameters (i.e. prothrombin time (PT) and size of RBCs (MCV)) did not indicate significant changes in the treatment groups. In total, it could be argued that the bulk group showed minimum hemocompatibility, while the carrier and NED groups appeared to have minimal effects on

hemocompatibility parameters. In other words, loading the drug in the NE (i.e. formation of NED) may increase the hemocompatibility of the drugs.

In vivo efficacy studies

To assess the anti-tumor performance of NED and bulk formulations, MRI was used to compare the differences between the tumor volumes as a function of the treatment groups. Brain tumors appeared irregular to roundish having a white border surrounding the white or gray area in T2-weighted MRI data (Fig. 4A). After 14 days of tumor implantation (i.e. beginning of the treatment), MRI scans were obtained in all rats with C6 cerebral gliomas to confirm the development of glioma tumors and examine the volume of tumors. The rats were then randomly divided into four groups for treatment. The tumor volume in animals during the study period was measured and is given in Fig. 4. At the start of the treatment, animals in the control group had a tumor volume with mean \pm SD tumor volume of 0.047 ± 0.021 cm³ and after 7 days, tumor volume grew up to 0.291 ± 0.129 . In the groups treated with free drugs (bulk), compared to the carrier and NED groups, tumors progressed faster after 7 days: the largest volume with mean \pm SD tumor volume of 0.500 ± 0.109 cm³ (71.8% growth compared to control group) was observed (Fig. 4B). Most of the animals in this group did not reach the next imaging time and died. However, in one animal, the tumor disappeared completely, and the animal survived up to 60 after the start of the treatment. In the carrier group, the growth rate of the tumors was slightly slower than the control group with mean \pm SD tumor volume of 0.134 ± 0.069 cm³ (54% inhibition growth) on day 7 and 0.234 ± 0.084 on day 21 of treatment. The tumor growth rate in the NED group

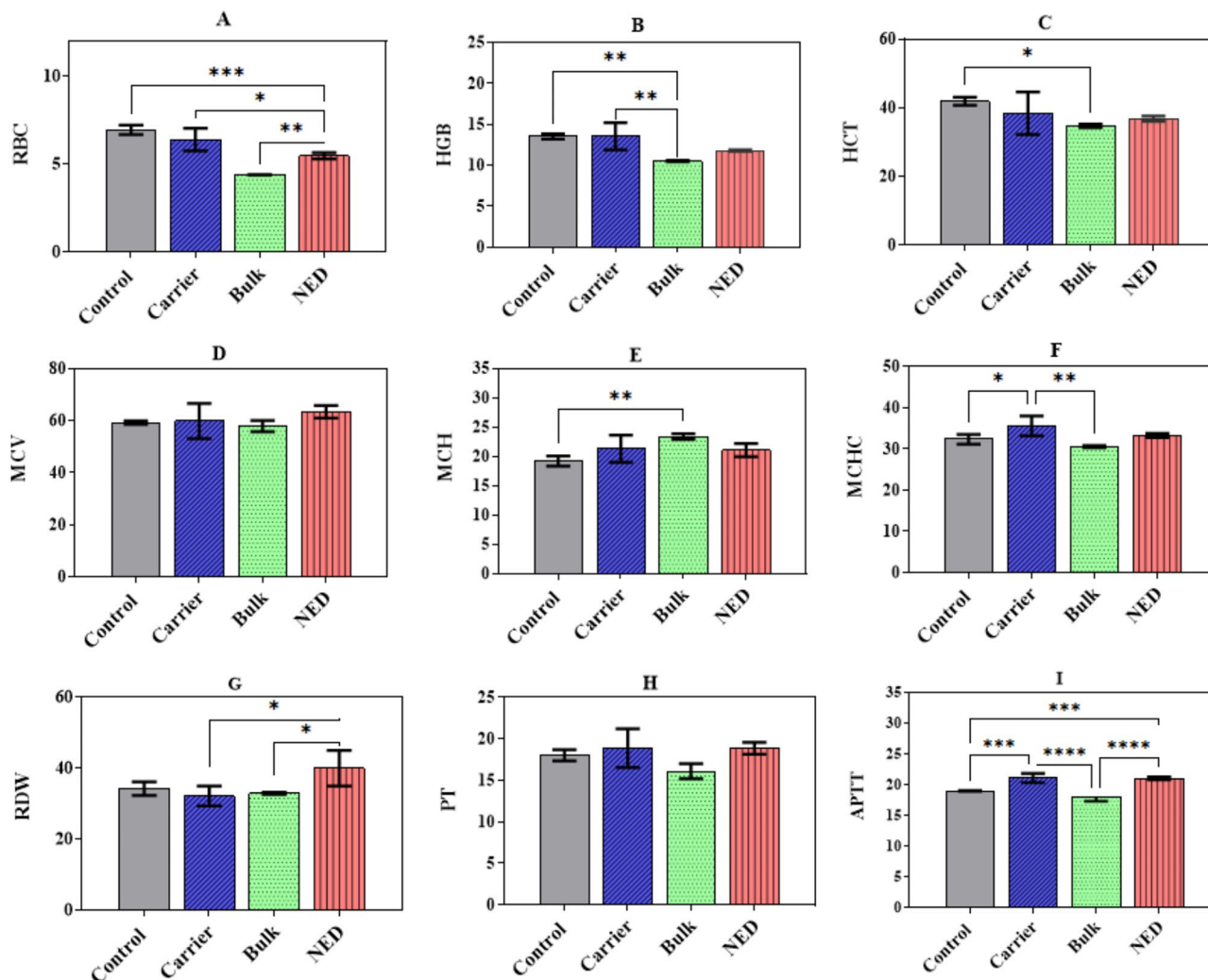


Fig. 3 Hemocompatibility changes after 4 injections in a twice-a-week regimen, compared with the control group (normal saline) in NED (nanoemulsion containing the drugs), bulk (THC and CBD), and carrier (nanoemulsion without the drugs): **(A)** RBC: the number of red blood cells, **(B)** HGB: the amount of Hemoglobin in blood, **(C)** HCT: the hematocrit test, measuring the proportion of RBC, **(D)** MCV: mean corpuscular volume, the average size of RBC, **(E)** MCH: the mean corpuscular hemoglobin, **(F)** MCHC: the mean corpuscular hemoglobin concentration, **(G)** RDW: red blood cell distribution width, **(H)** PT: prothrombin time used to evaluate blood clotting, **(I)** PTT: partial thromboplastin time used to measure how long it takes to make a clot. *P*-value annotation legend: **p* < 0.05, ***p* < 0.01, ****p* < 0.001 and *****p* < 0.0001

was slower than the other groups (0.072 ± 0.040 with 75% inhibition growth and 0.402 ± 0.136 cm³ on days 7 and 60 post treatment). One animal in the NED group induced tumor regression, and during the treatment period, the tumor disappeared completely.

Survival rates of glioma-bearing rats are illustrated in Fig. 5. The median survival of the control and bulk groups was 9 and 4 days after the start of treatment, respectively. While this value improved slightly in the carrier group (i.e. 12.5 days). Also, there was a significant change in the survival rate of animals in the NED group (i.e. 51 days).

Furthermore, at this time point, we observed that during the period of receiving the NED, the appetite of animals increased temporarily (~6 h). Besides, in the NED group, the animals were in better condition in terms of

their appearance and weight. Bleeding from the ear and nose appeared 30 days after the start of the treatment in the NED group, 6 days in the control groups, 4 days in the Bulk group and 12 days in the carrier group. The results of Log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon analyses are presented in Table 1.

Discussion

In this study, we prepared a nanoemulsion of cannabis extract using a titration method by combining surfactants and a co-surfactant at constant weight ratios. We reported a stable NE with no sign of phase separation. By enhancing the solubility of drugs, especially in the case of poorly water-soluble ones, NEs have indicated potentials for different administration routes, including topical [18,

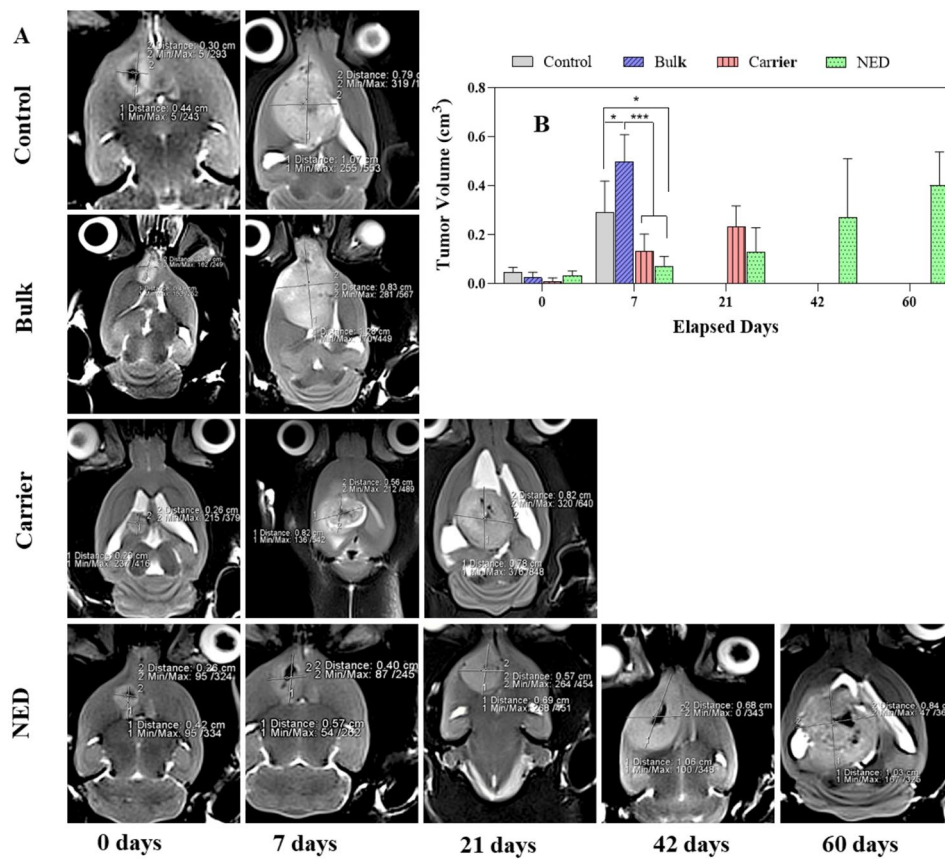


Fig. 4 (A) Examples of MR tumor images on coronal plane and its growth in groups treated with NED (nanoemulsion containing the THC and CBD), bulk (THC and CBD), and carrier (nanoemulsion without drug), (B) The diagram of tumor volume growing related to each group over elapsed days. *, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

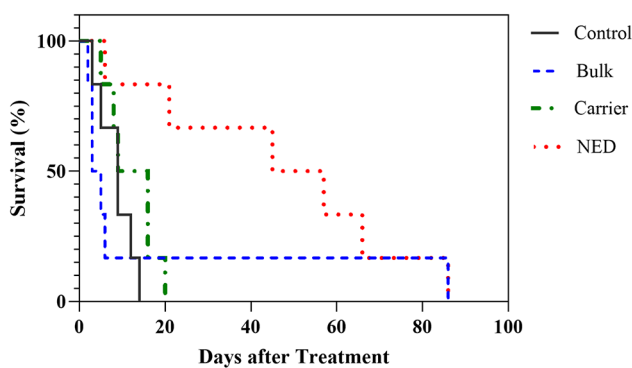


Fig. 5 The Kaplan-Meier survival curve of rats with C6 cerebral glioma treated with NED (nanoemulsion containing the THC and CBD), bulk (THC and CBD), and carrier (nanoemulsion without drug)

19], oral [20] and inhalation [21]. The surfactants and co-surfactants in NEs aid in modifying the permeability of the drug [22–24].

To construct platforms for improved delivery of medicines to the brain, it is crucial to understand the factors that limit drug transport across the BBB. Lipid nanoparticles are absorbed by adsorptive-mediated endocytosis, a method for penetration through the BBB. Additionally,

Table 1 The log-rank method and Gehan-Breslow-Wilcoxon analysis for survival data ($n = 6$)

Groups	Log-Rank method P-value	Gehan-Breslow-Wilcoxon P-value
Control vs. Bulk	0.5789 (ns)	0.2218 (ns)
Control vs. Carrier	0.1482 (ns)	0.2937 (ns)
Control vs. NED	0.0072 (**)	0.0180 (*)
Bulk vs. NED	0.1953 (ns)	0.0444 (*)
Carrier vs. NED	0.0102 (*)	0.0290 (*)

passive diffusion is used to transfer small nanoparticles [1, 25].

In our study, the hydrodynamic diameter of the NED was 12.7 ± 1.2 nm, which is ideal for most drug delivery systems. The optimal size of nanoparticles for crossing the BBB is less than 20 nm, as larger particles are not able to cross the tight junction [26]. The zeta potential of the optimized NED was close to zero (-0.1 ± 0.1 mV), showing that steric stabilization by nonionic surfactants (i.e. Span 80 and Tween 80) was the main stabilization phenomenon [27].

We also examined hemocompatibility factors (RBC, MCV, MCH, MCHC, RDW, PPP, PT and PTT) to test the

potential in vivo toxicity to rat blood. Generally, in the majority of people receiving chemotherapeutic agents, hematological blood factor levels, such as RBC WBC, and platelet count, decrease. Changes in blood factor levels are mostly a consequence of the mechanism of action of the anticancer drug [28]. Cannabis has been reported to cause important effects on blood factors. A sharp decrease in the WBC, MCH, RDW, MCV and MPV has been reported for cannabis, which causes anemia with iron deficiency [29]. When comparing the Bulk cannabis extract with the nanoemulsion, our nanoformulation improved the hemocompatibility of the extract on several blood parameters. This could be due to direct contact between the active ingredients which occurs when the active ingredients are in bulk form. In a study, the combination of CBD and THC induced the generation of reactive oxygen species (ROS), which damaged the RBC membrane and caused the degradation of hemoglobin. RBC deformities can eventually prevent oxygen delivery to tissues, resulting in hypoxia [30]. An increase in the bioavailability and efficacy of the active ingredient has been reported to reduce the required dose of the drug [31], thus, may contribute to lower toxicity of the nanoformulation.

A cytotoxicity study of the different groups on rat glioma cell lines in our study showed that the highest cytotoxicity was in the carrier group, while the bulk group showed no toxicity. It is arguable that in cell culture, the bulk group is not miscible with the cell culture medium due to its hydrophobicity, resulting in lower cytotoxicity compared to the NE forms. On the other hand, when NED and carrier groups were mixed with the cell culture medium, it was visually observable that the vesicle structure was unstable (i.e. phase separation was observed). Therefore, as has been discussed previously [12], we believe that some NED droplets tend to separate into two phases and form a creamy layer on top of the culture medium. Consequently, the released surfactants interact with the cells, increasing the cytotoxicity of the preparation.

To evaluate the antitumor properties of the different treatments, the presence and volume of tumors in the treatment groups were assessed using MRI. Tumors grew faster and more aggressively in the bulk group, compared with the NED group. This progressive tumor growth in the bulk group may be due to the hydrophobic nature of CBD and THC. The tiny size of cannabis drugs in NE form leads to increased bioavailability [32], thus, improved in vivo performance. While early research indicates that cannabinoids may have systemic therapeutic effects and can exert anti-cancer effects in cell culture models, results can be variable depending on tumor types, specific cannabinoids used, dosages, and their delivery methods [33–36].

The primary objective of this study is to investigate whether the development of novel nanoemulsions incorporating THC and CBD can enhance their anticancer properties compared to their unformulated counterparts. The formulation process involves creating NE with excipients to improve drug solubility and absorption. In cell culture experiments, we found that the NE as a carrier exhibited greater cytotoxicity, likely due to the presence of surfactants, while the combination of CBD and THC appeared to reduce cytotoxicity. Additionally, the in vivo studies yielded compelling results. Although the NE free cannabinoids and NED both significantly reduced tumor growth and animals treated with the NED demonstrated improved overall survival compared to those receiving the free cannabinoids (NE). The increased survival rate is a crucial outcome in the treatment of cancerous tumors throughout the body. Advances in medical research, targeted therapies, and early detection methods have significantly contributed to improving patient prognosis and overall survival rates [37–39].

Our findings also showed that the NE containing the drug (i.e. NED) could interrupt tumor progression, which is arguably due to increased penetration into the gliomas cells. Increased delivery of the active ingredients to the brain has been already shown for NEs [22–24]. NEs can have a longer biological life in the blood compared with the free form of the drug, thus increasing accessibility of the active ingredient(s) to the tumors [32, 40]. Additionally, Tween appears to inhibit glycoprotein P, which is responsible for recycling foreign substances from BBB into the bloodstream and can play a role in damaging tumors [41]. Other studies have also shown the effectiveness of NEs in drug delivery. Valproic acid, loaded in NE, was reported to be more effective than its bulk form. Additionally, it indicates low toxicity and high bioavailability in the brain, which was due to improved crossing of the BBB [40]. NE containing CBD for human corneas also demonstrated enhanced anti-inflammatory effects and wound healing properties as well as decreased toxicity compared to those of the bulk group. Furthermore, a six-fold increase in the residence time of the active ingredient was reported on the corneal surface, indicating the positive effect of NE formulation on the prevention of degradation and metabolization of CBD [42].

Conclusion

Based on our findings, the nanoemulsion model containing CBD and THC increased the antitumor effect of the drugs. This may be due to the role of nanoemulsions in improving drug delivery across the blood-brain barrier and improving blood compatibility during intravenous drug administration. However, this study is a primary investigation in the rat animal model, and future studies

should consider further evaluation of toxicity and efficacy in larger animal populations.

Abbreviations

NE	Nanoemulsion
THC	Δ^9 -Tetrahydrocannabinol
CBD	Cannabidiol
LNC	Lipid nanocapsule
NED	Nanoformulation containing THC and CBD
RBC	Red blood cells
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
RDW	Red cell distribution width
PPP	Platelet-poor plasma
PT	Prothrombin time
PTT	Partial thromboplastin time
DLS	Dynamic light scattering
MRI	Magnetic resonance imaging

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40360-024-00788-w>.

Supplementary Material 1

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Not applicable.

Author contributions

HME: Writing—original draft; Conceptualization; Data curation; Formal analysis; Investigation. FH: Formal analysis; Investigation, Writing—review & editing. FE: Methodology, Writing—review & editing. SAS: Methodology, Writing—review & editing. HG: Conceptualization, Methodology, Writing—review & editing. AA: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Writing—review & editing.

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Data availability

The supporting information file provides data on the manuscript. More detailed data are available upon request.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of North Khorasan University of Medical Sciences (IR.NKUMS.REC.1400.112).

Consent for publication

The authors give their consent for the publication of identifiable details, including the images within the text to be published in the journal and article.

Competing interests

The authors declare no competing interests.

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