International Journal of Biological Macromolecules 88 (2016) 320–332

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Quantification and evaluation of thymoquinone loaded mucoadhesive nanoemulsion for treatment of cerebral ischemia

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ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form 7 March 2016
Accepted 9 March 2016
Available online 11 March 2016

Keywords:
Thymoquinone
Cerebral ischemia
UPLC-PDA method validation
Nanoemulsion
Brain pharmacokinetics

ABSTRACT

Stroke is an important cause of deaths worldwide, resulting in an irreversible deterioration of the central nervous system. Finally, production of more free radicals. Therefore, Thymoquinone is having antioxidant property and reported to have a potential role in the amelioration of cerebral ischemia but due to low solubility and poor absorption; they exhibit low serum and tissue levels. Present work aims to prepare nanoemulsions in order enhance the bioavailability of drug and hence evaluate the drug targeting in brain via non-invasive nasal route administration. Thymoquinone Mucoadhesive Nanoemulsion (TMNE) was prepared by ionic gelation method; characterized for particles size, entrapment efficiency, zeta potential, and ex vivo permeation study. Optimized TMNE ended up with a mean globule size 94.8 ± 6.61 nm; zeta potential −13.5 ± 1.01 mV; drug content 99.86 ± 0.35% and viscosity 110 ± 12 cp. Ultra Performance Liquid Chromatography-Photodiode Array (UPLC-PDA) based bioanalytical method was developed and validated for pharmacokinetics, biodistribution, brain-targeting efficiency (628.5786 ± 44.79%) and brain drug-targeting potential (89.97 ± 2.94%) studies via post intranasal administration which revealed enhanced bioavailability of TQ in brain as compared to intravenous administration. Improved neurobehavioural activity (locomotor and grip strength) was observed in middle cerebral artery occlusion induced cerebral ischemic rats after i.n. administration of TMNE.

1. Introduction

Despite remarkable advances in the prevention and treatment of cerebral ischemia or stroke, a leading cause of death and disability in the aged population [1–3]. Forebrain ischemia induces complete interruption of blood flow, producing inadequate delivery of oxygen to brain tissue and leading to a decrease in glucose utilization and adenosine triphosphate (ATP) production. Failure of energy production and depleted ATP leads to lactic acidosis, causing a major rapid redistribution of ions across plasma membrane. As a result, there is depolarization of neurons and glia [4], triggering excessive release of glutamate into the extracellular space. This leads to overstimulation of glutamate receptors, inducing marked expression of several pro-oxidant enzymes or mediators [5].

Oxidative stress is one of the primary factors that exacerbate damage by cerebral ischemia [1,6]. Several components of reactive oxygen species (superoxide, hydroxyl radical, hydrogen peroxide and peroxynitrite radical) generated after ischemia–reperfusion injury play an important role in neuronal loss after cerebral ischemia [1,6,7]. Brain tissues are particularly susceptible to oxidative damage; therefore, it is believed that pharmacologic modification of oxidative damage is one of the most promising avenues for stroke therapy.

A number of antioxidants drugs (thioperamide, ropinirole, curcumin) are reported to reduce ROS-mediated reaction sand rescue the neurons from reperfusion-induced neuronal loss in animal models of cerebral ischemia [1–3,8]. Recently, in vitro and in vivo studies confirmed the use of Thymoquinone in cerebral ischemia which is chiefly found a major component in *Nigella sativa* seeds [9,10]. Thymoquinone (TQ), the main constituents of the volatile oil

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http://dx.doi.org/10.1016/j.ijbiomac.2016.03.019
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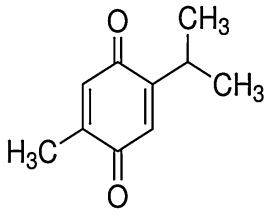

Fig. 1. Chemical structure of Thymoquinone.

from *N. sativa* seeds, is reported to have potential role as a strong antioxidant [9,10].

Thymoquinone (Fig. 1) is a liposoluble benzoquinone-based phytochemical with a remarkable antioxidant and anticancer activities [11]. Thymoquinone exhibits low serum and tissue levels due to its poor solubility, absorption, extensive metabolism, and rapid elimination [12]. To enhance the bioavailability of thymoquinone, various strategies have been applied in terms of formulations and route of administration. The interesting area in this regard is to bypass Blood–brain barrier (BBB) and thus preferentially target the brain in order to treat neurological disorders [13]. Intranasal administration of thymoquinone can be an effective way, to bypass blood–brain barrier, transport more drug and achieve much higher degree of drug concentration in the cortex, caudate-putamen, and hippocampus as compared to intravenous administration [14]. The design and development of new drug delivery systems with concept, to enhance the efficacy of existing drugs is an ongoing process in pharmaceutical research [15].Therefore, producing suitable formulations in order to improve the solubility and bioavailability of such drugs is very necessary. The lipid based formulation approach gained wide attention in order to enhance drug uptake in the CNS [1–3]. Thus, we investigated a formulated Nanoemulsion loaded with Thymoquinone through intranasal administration in middle cerebral artery occlusion-induced focal cerebral ischemia in Wistar rats. Nanoemulsions with a size range (10–200 nm) exhibited more stability in suspension due to small particle size [16]. The fact that, nanoemulsions have a remarkable small oil droplets size, it, transparent appearance and weak light scattering properties, makes them suitable to be incorporated into optically transparent products without adversely affecting their clarity [17]. Furthermore, nanoemulsions with intranasal route of administration and targeted brain drug delivery are attractive candidates for improving drug solubility, reducing side effects of various potent drugs, and prolonging the pharmacological effects in comparison to conventional formulations such as conventional emulsions [18,19]. The objective of current work is to prepare, characterize and target CNS by thymoquinone loaded mucoadhesive nanoemulsion (TMNE) in order to treat the cerebral ischemia. One of the most important parameters of targeting efficiency of drug in the brain is also calculated.

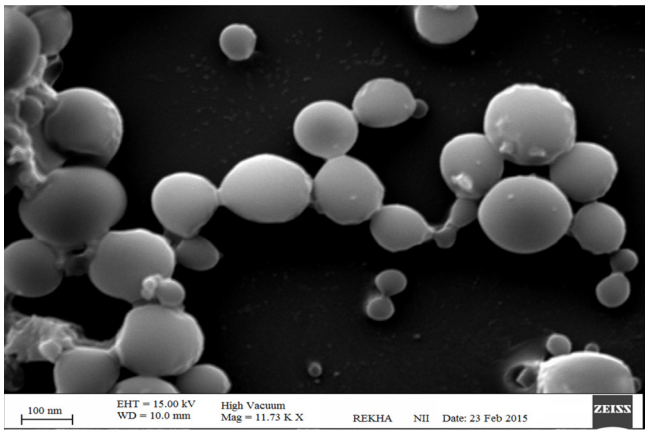

Fig. 3. Scanning electron microscopy images (SEM) of surface morphology of the prepared Thymoquinone Mucoadhesive Nanoemulsion.

2. Materials and methods

THQ (Sigma-Aldrich, St Louis, MO, USA), Ammonium formate were obtained from Fluka analytical (Sigma-Aldrich, the Netherlands). Formic acid (Assigned purity > 98%) was obtained from Fluka analytical (Steinheim, Germany). Deionized water was purified using Milli-Q water purification system (Millipore, Bedford, MA, USA). Gift samples of oleic acid, carbitol, Tween20 were kindly supplied by Wockhardt Research Centre, Aurangabad, India. Methanol high-pressure liquid chromatography (HPLC) grade and

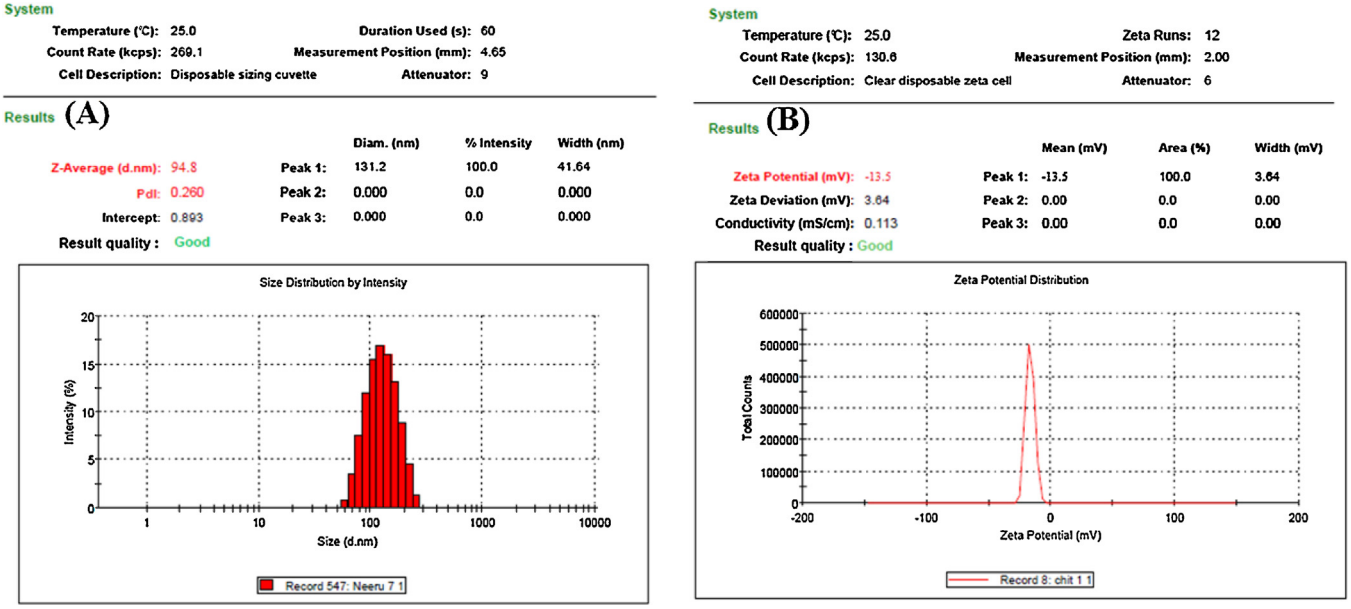

Fig. 2. Dynamic light scattering techniques for determining the particle size distribution of Thymoquinone Mucoadhesive Nanoemulsion Globule Size (A), and zeta potential of Thymoquinone Mucoadhesive Nanoemulsion (B).