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Development water in oil nanoemulsion of diethylcarbamazine for enhanced the characteristics for lymphatic targeting: A proof of concept study

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ABSTRACT

As an antifilariasis drug, oral administration of diethylcarbamazine (DEC) could not effectively deliver the drug to the lymphatic system. Hydrophobic formulation with a particle size of <100 nm could improve the delivery of drug. Accordingly, we developed water in oil nanoemulsion encapsulating DEC. The nanoemulsion was less than 100 nm with negative charge, showing its suitability for lymphatic targeting. The nanoemulsion could sustain the release of DEC and improve the retention in intestinal tissue in comparison with DEC solution. Importantly, this approach did not cause any hemolysis in *in vitro* study and any irritation in *in vivo* study.

1. Introduction

According to data from the WHO in 2010, more than 120 million people in 83 countries were infected with lymphatic filariasis (LF), of whom about 40 million have been disabled or paralyzed by it. Its distribution extends from Latin America, across central Africa, southern Asia, and into the Pacific islands [1]. Furthermore, WHO has reported that around 51 countries still require LF treatment using mass drug administration (MDA). As one of drugs used in MDA, diethylcarbamazine (DEC) been used through oral administration with a dose of 6mg/KgBB/day for 12 days [2].

In LF disease, the adult filarial stay in the lymphatic system. Therefore, it is important to ensure that the drug is delivered in the lymphatic system for effective therapy. In order to reach the lymphatic system, drugs administered orally must have a good lipophilicity and log P value of >4.7 [3]. Nevertheless, DEC is hydrophilic drug with log P value of 0.3. With this in mind, it is unlikely that oral administration could not deliver DEC to the infection site. Our previous study showed the limited concentration of DEC after conventional oral administration. Therefore, an effective drug delivery system is needed in order to reach the target of the lymphatic system for effectiveness of the treatment of LF.

Several delivery systems have been developed to improve the delivery of drugs to the lymphatic system. However, most of the methods require complicated technique with sophisticated instrument. Nanoemulsion, especially water in oil type, was considered as the appropriate system due to the high lipophilicity of oil used in the oil phase, which can be prepared by simple method. Nanoemulsion is one of the types of emulsion that have a drip that can reach less than 500 nm [4]. The nanoemulsion water-in-oil (W/O) is a type of emulsion with nanometric droplets that surfactant made the droplets dispersed in the oil phase [5,6]. Nanoemulsion can increase the permeability and retention effects on the target tissue [7]. Nanoemulsion has been reported to be absorbed directly into the lymphatic system and can avoid the metabolism of the first line of the liver so that the drug's bioavailability increases [8]. Considering all these backgrounds, here, for the first time, we developed W/O nanoemulsion containing DEC for improved treatment of LF. The nanoemulsion was characterized for their physical properties. Specifically, the *ex vivo* intestinal permeation study was investigated. Finally, *in vitro* toxicity and *in vivo* intestinal irritation were evaluated.

2. Material and methods

2.1. Formulation and screening of water in oil nanoemulsion

The nanoemulsions were prepared using simple homogenization method. Tween®80 and Span®80 were used as stabilizers for water and

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Table 1
Nanoemulsion water in oil formulation (%w/v).

Formulation	DEC	VCO	Water	Tween 80	Span 80	Glycerin
F1	1%	60%	9%	10%	10%	10%
F2	1%	60%	9%	12%	8%	10%
F3	1%	60%	9%	8%	12%	10%
F4	1%	55%	9%	12.5%	12.5%	10%
F5	1%	55%	9%	15%	10%	10%
F6	1%	55%	9%	10%	15%	10%

oil phases, respectively. The water phase was poured into the oil phases under homogenization using Ultra Turrax for 3000 rpm for 15 min. All formulations were tested for their emulsion type using Sudan III and short stability using centrifugation for 3000 rpm for 15 min to identify its stability. All formulations contained 1% w/v of DEC in water phase.

2.2. Characterization of nanoemulsion

Malvern instruments were used to measure the particle size, polydispersity index, and zeta potential. Nanoemulsion was observed with TEM to identify its morphology. Samples were sealed in aluminum DSC pan and scanned in the temperature range of 0–300 °C [9]. FTIR was used to detect the present functional groups of pure DEC and DEC in nanoemulsion at 400-4000 cm^{-1} [9].

2.3. *Ex vivo* intestinal permeation and retention study

Ex vivo permeation studies were carried out in the diffusion of Franz cells. The intestinal tissue of Wistar rat was used in this study. Furthermore, the amount of DEC localized in the intestinal tissue after 1 h, 4 h, and 8 h of *ex vivo* permeation study was also determined.

2.4. *In vitro* hemolysis study, *in vivo* irritation and histopathological studies

In vivo irritation evaluation was carried out in Wistar rats (n = 4). All *in vivo* studies were approved by Ethical committee of Faculty of Medicine, Hasanuddin University, Indonesia. In this study, all rats were divided in three cohorts, namely blank nanoemulsion, nanoemulsion containing DEC and distilled water treated groups. All samples were administered orally for 3 days. In the end of experiment, rats were ethically sacrificed. The intestines and stomach were excised for further histopathological assessment with hematoxylin-eosin (HE) staining.

3. Result and discussion

3.1. Formulation and screening of water in oil nanoemulsion

This study aimed to encapsulate DEC into W/O nanoemulsion. In order to form W/O emulsion, it was crucial to consider the selection of the basis of formulation. Therefore, in this study, the percentage of oil phase was used in the concentration of more than 50%. Importantly, the

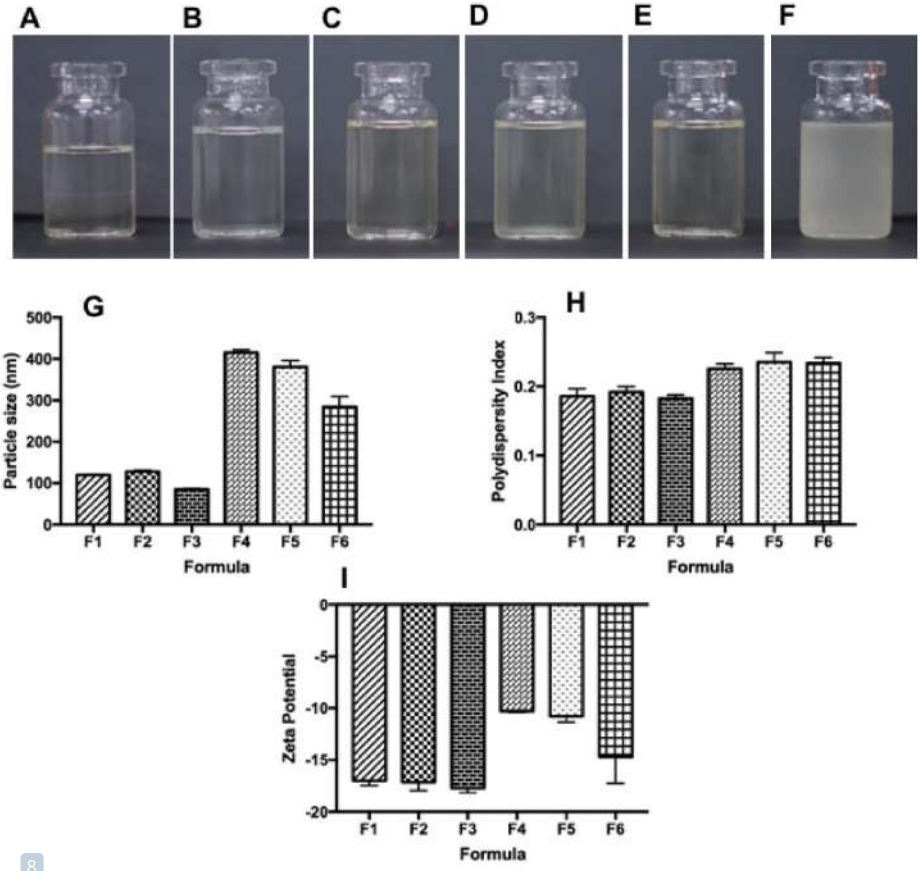

Fig. 1. Images of F1 (**A**), F2 (**B**), F3 (**C**), F4 (**D**), F5 (**E**) and F6 (**F**). Particle size (**G**), PDI (**H**) and zeta potential (**I**) of nanoemulsion.

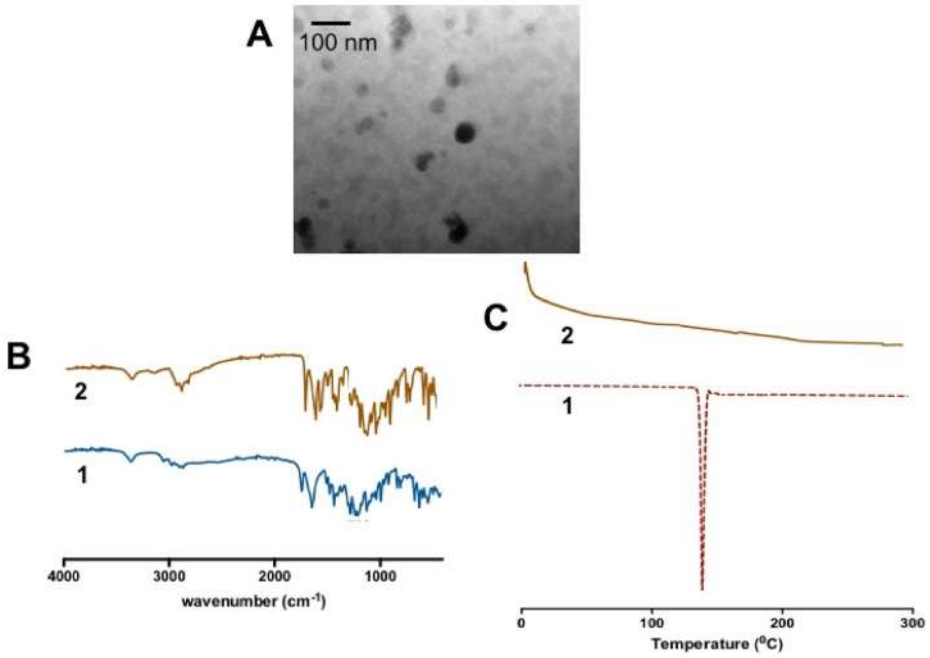

Fig. 2. TEM image of droplet in water in oil nanoemulsion (**A**). FTIR spectra (**B**) and DSC thermograms (**C**) of DEC (1) and nanoemulsion (2).

combination of Tween 80 and Span 80 has been reported to form W/O emulsion. Furthermore, glycerin was used to stabilize the emulsion. Following various screening processes, six formulations were selected due to their ability to form stable emulsion after centrifugation process and the formation of water in oil emulsion (Table 1). Furthermore, Fig. 1 shows the representative images of six nanoemulsion.

3.2. Characterization of nanoemulsion

The selected formulations were further characterized for their size, PDI and zeta potential. The use of the non-ionic surfactants was able to stabilize the nanoemulsion due to their capabilities to show high adsorption at the interface of the oil-water [10,11]. It was shown that the increase of Span 80 concentration, the particle size decreased. It might be due to the interaction of the electrical repulsive occurring between the surfactants which was then attached into the surface of the emulsion droplets [12,13]. It has been previously reported that due to the specific characterizations of the lymphatic system, particles below 100 nm are easily absorbed by the lymphatic system. Therefore, F3 with particle size of 84.67 ± 2.08 nm would be a great candidate for this purpose. Zeta potential is one of the crucial parameters to evaluate the stability of the nanoemulsion. It can be used to confirm the results of the particle size and describing the degree of aggregation of the internal phase of the nanoemulsion [10] Furthermore, it has been reported that in the nanoemulsion formulation, the zeta potential values of between −20 mV and −3 mV were found to be kinetically stable [14]. In this study, it was found that all formulations possessed negative charges (between −17 mV and −6 mV), which was beneficial for the lymphatic transport. Accordingly, it could be confirmed that the formulations developed in this study showed the slow rate of coalescence [10]. Particles with negative charges has been found to easily leave the interstitial tissue and be absorbed by the lymphatic system. The particle size data were supported by TEM (Fig. 2A), showing that the diameters of droplets were less than 100 nm. Additionally, the interfacial tension of the surfactant is one of the vital characteristics in the interfaces of fluid-fluid and emulsions. The correlation between the characteristics and the emulsification formation and stability has been reported in several studies [15]. Moreover, it has also been found that the use of glycerol could decrease the interfacial tension of the emulsion, thereby improving the stability of the formulation. Importantly, the interfacial tension of the emulsion is also affected by the type of surfactant [16]. Different concentrations of Tween 80 (hydrophilic surfactant due to the presence of POE chains) and Span 80 (lipophilic sorbitane monooleate surfactant) influenced the dynamic interfacial tension between water and mineral oil in the emulsion formulation. This might be due to the instable rate spreading of surfactant on the surface of the internal phase, which is associated with the mechanism of adsorption and desorption of the molecules of surfactants [15,16].

Fig. 2B shows the thermogram of DEC and nanoemulsion of DEC. The thermogram of DEC showed an endothermic peak at 166 °C, presenting the melting point and the crystallinity of DEC. Meanwhile, in nanoemulsion profile, there was no peak found, indicating that DEC contained in the nanoemulsion was no longer in crystalline form but changed into an amorphous form.

In the FTIR spectrum, it was shown that several peaks were observed in DEC, namely 1410 cm^{-1} (C–C stretching), 1623 cm^{-1} (C = 0 stretching), and 3048 cm^{-1} (C–H stretching). All these peaks appeared in nanoemulsion spectrum. This indicates that DEC contained in the nanoemulsion did not interact with other ingredients.

3.3. Ex vivo permeation and retention study

The controlled release pattern was observed in *ex vivo* intestinal permeation study (Fig. 3A). It is important to bear in mind that prior to the absorption in the lymphatic system, drug should be able pass through intestines epithelium. Nanoemulsion was successfully able to help and facilitate the permeation of DEC through intestinal tissue, while controlling the release over 8 h. Importantly, significant amount of DEC were retained in the intestinal tissue following the administration of nanoemulsion, which was 1.083 μg/g intestinal tissue, compared

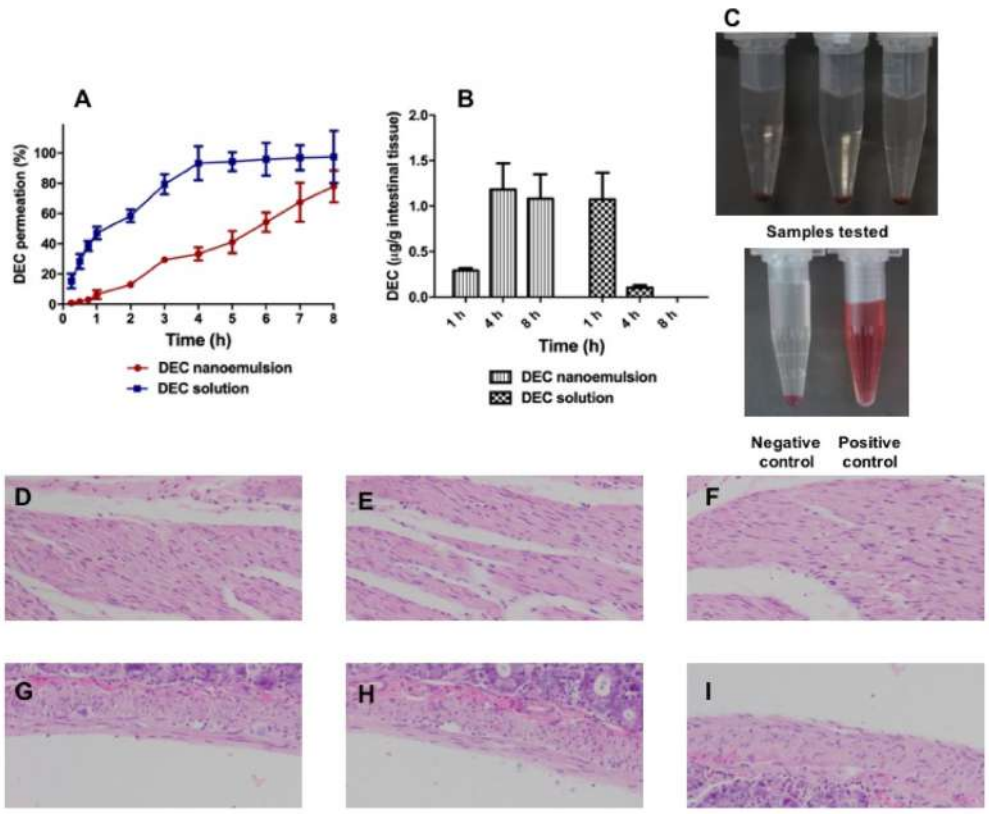

Fig. 3. *Ex vivo* permeation (**A**) and retention (**B**) of DEC from water in oil nanoemulsion and control solution through intestinal tissue (mean ± S.D., $n = 3$). Images of hemolysis study (**C**) Histopathological cross-section of the rats' stomach after oral administration of water (**D**), blank nanoemulsion (**E**) and DEC nanoemulsion (**F**). Histopathological cross-section of the rats' intestinal after oral administration of water (G), blank nanoemulsion (**H**) and DEC nanoemulsion (**I**).

to DEC solution, showing that no DEC detected in the intestinal tissue after 8 h (Fig. 3B). The amount of DEC retained in the intestinal tissue could potentially permeate through and reach the lymphatic system. It has been reported that the transportation of the nanoparticle formulation to the targeted site is affected by their environment [17,18]. Therefore, in the further study, it is crucial to evaluate the stability of the nanoemulsions in the intestinal tissue to ensure that the nanoemulsions still possess the similar characteristics. Therefore, the transportation to the lymphatic system would not be afftected.

3.4. *In vitro hemolysis study, in vivo irritation and histopathological studies*

In vitro toxicity was assessed by hemolysis activity. Significantly, the hemolysis activity values were less than 5% at all tested concentrations, as presented in the Fig. 3C. Therefore, the results indicated that this approach would be safe to use.

Finally, *in vivo* irritation was assessed after 3 days oral administration and was evaluated by observing the histopathology of the gastrointestinal mucosa of rats. Importantly, the pathology of the gastric and intestinal mucosa of rats showed that there was no evidence of irritation of the gastric mucosa and disturbance of the intestinal villi (Fig. 3D–I). The results obtained here showed the great potential of nanoemulsion as delivery approach to deliver DEC to the lymphatic system. Further studies regarding *in vivo* lymphatic delivery and pharmacodynamic in an appropriate animal model should be conducted.

4. Conclusion

In the present study, we developed nanoemulsion containing DEC using Tween 80 and Span 80 as the surfactanss. Moreover, glycerol was used to improve the stability of the nanoemulsion. High concentration of oil phase was selected to form water in oil emulsion to meet the criterion of the lymphatic targeting. With respect to the results of the characterization evaluations, the formulation fulfilled the physical requirement for the lymphatic targeting with particle sizes of less than 100 nm and negative charge. Furthermore, the incorporation of DEC into nanoemulsion was able to sustain the release of DEC over 8 h. FTIR study exhibited no chemical interaction between DEC and all excipients used in this study. Importantly, the formulations was found to be non-toxic and non-irritant in the gastrointestinal tissue.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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