

IN VIVO CHARACTERIZATION OF LESS PAINFUL PROPOFOL NANOEMULSION USING PALM OIL FOR INTRAVENOUS DRUG DELIVERY

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ABSTRACT

Objective: The objective of present work was to evaluate the effectiveness of propofol in nanoemulsion-based palm oil that called as NEMS™, which was a choice of anesthetic drug to induce and maintenance general anesthesia to reduce pain on injection activity and also to evaluate the *in vivo* characterization of propofol in NEMS™.

Methods: Preparation of propofol nanoemulsion using NEMS™ technology has been performed for propofol 1% in NEMS™ (P1%), and propofol 2% in NEMS™ (P2%). Determination of free propofol concentration in the aqueous phase was conducted using HPLC and rat paw lick test was evaluated as *in vivo* test to assay the intensity of pain on injection site. The sleep recovery test was conducted to evaluate the pharmacological effect and erythrocyte hemolysis test also conducted to ensure the safety of propofol in NEMS™. All of the test results were compared with Diprivan®1% as a positive standard.

Results: The contents of free propofol in formulation P1% and Diprivan®1% in aqueous-phase were 6.20±0.03 µg/ml and 15.02±0.33 µg/ml, respectively (*P<0.05). The rat paw lick test showed that the formulation P1% was significantly (*P<0.05) less painful when compared to Diprivan®1%. There were no significant differences in pharmacological effect for all of the formulations (*P>0.05). The erythrocyte haemolysis test shows that all formulation was still safe for our blood.

Conclusion: Palm oil can be used as a carrier for propofol and it was successfully reduced the free propofol contents and the intensity of pain on injection site in rats.

Keywords: Palm oil, NEMS™, Propofol, MCT/ICT, Nanoemulsion

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INTRODUCTION

Propofol nanoemulsion using soya as the oil phase has been used for decades in clinical application. This drug is widely used in surgery procedure and also generally used in an intensive care unit all around the world. Perhaps, this is the reason why it becomes one of the popular anesthetic agents [1]. So far, this dosage form is reputed success because it has a rapid onset of action, short duration of action and minimum side effects [2].

Unfortunately, it was found so many disadvantages from this dosage form. Pain of injection is one of the serious problems that often happen to the patients [3-5]. Many researchers have tried to find the solution how to reduce the pain such as by using rapid injection technic [6], utilizing the analgesic drug before injection, such as lidocain [7], ephedrine [8], saline dilution [9], paracetamol [10], lornoxicam [11], etc. However, the best solution to treat the pain has not been discovered [4]. Further studies are needed to make an effective formulation.

The clear mechanism why there is pain on site following injection is still not well understood. Some researchers make an assumption that the free propofol in the aqueous phase of propofol nanoemulsion is known to be related with the intensity of pain at the injection time [12]. The utilization of propofol nanoemulsion using a mixture of medium chain triglyceride (MCT)/long-chain triglyceride (LCT) to reduce the intensity of pain has become one of the safety solutions to be used [3, 13-15]. Yamakage *et al.* [14] stated that MCT/ICT propofol nanoemulsion reduces the concentration of free propofol until 30-35% so that it will reduce the pain.

In this study, palm olein oil (POO) as the source of palm oil was used instead of soya oil as an oil phase in propofol nanoemulsion preparation. This oil is easily to find and commonly people use it as cooking oil, in margarine preparation and as an additive for food

preparation [16, 17]. However, the application of this oil in pharmaceutical was still uncommon although this oil has the potentiality to be developed. Production of this oil every year is very high. This oil came originally from West Africa and it was introduced to Brazil and other tropical countries in 15th century. Currently, Malaysia and Indonesia are two countries with the highest production of palm oil in the world [18].

In the present study, the comparison in intensity of pain from P1% and P2% with Diprivan®1% was evaluated by determination of free propofol concentration in the aqueous phase by HPLC method. The rat paw lick test was performed to compare the intensity of pain in animals after injection. The sleep recovery test was conducted to compare the pharmacological effect of the formulations to Diprivan®.

MATERIALS AND METHODS

Materials

The following materials were obtained from the sources in brackets. Pure Propofol and Lipoid E-80 (GmbH, Ludwigshafen, Germany), Malaysian local palm oil for source of palm olein oil (POO) and MCT oil (Enersos, PharmaD Sdn. Bhd, Malaysia), Glycerol (Sigma Aldrich, Germany). Diprivan® was supplied by Astra Zeneca Macclesfield, UK. Double distilled water was used for all preparations. Chemicals for high-pressure liquid chromatography (HPLC) were HPLC grade and all other chemicals were analytical grade.

Methods

Preparation propofol nanoemulsion in NEMS™

The preparation of nanoemulsion was adopted from Prasetyo, *et al.* [19]. The nanoemulsion was prepared using the mixture of POO and MCT oil (1:1). High-pressure homogenizer was chosen as the method

to produce the nanoemulsion. POO, MCT oil, propofol (1% and 2%) and all ingredients that dissolved in oil were mixed in oil phase at 70 °C. The water-soluble ingredients were mixed in the aqueous phase using the same temperature. Then, the mixture was homogenized by using homogenizer at 10,000 rpm for 6 min. The final nanoemulsion was prepared by using high-pressure homogenization methods at 600 bar and 8 cycles. All of preparation processes were done aseptically in a clean room to ensure the sterility of nanoemulsion product.

Determination of free propofol concentration in aqueous phase

The determination method was adopted from Schicher *et al.* [20]. Propofol nanoemulsion (8.9 ml) in optiseal tube (Beckman coulter, USA) was centrifuged at 30,000 rpm and 4 °C for 5 h. The aliquot (10 µl) was taken using syringes and injected to the HPLC system to determine the concentration of free propofol contents. The HPLC analysis was performed with Waters system. The HPLC system was composed of a controller (Waters 600), a degasser AF, an autosampler (Waters 2707), and a photodiode array detector (Waters 2998). The separation was achieved with a reversed phase C18 (25 cm×4.6 mm; 5 µm particles) column. The mobile phase consisted of methanol and deionised water. It was sonicated for 15 min and then filtered through a 0.45 µm membrane filter paper. The wavelength use was 276 nm. Chromatography analysis was performed at ambient temperature (20±2 °C).

Animals

Male Sprague-Dawley rats weighing 300±20 g were obtained from Animal House of University Kebangsaan Malaysia. All rats were acclimatized to their environment for 1 w before the test. All rats were kept in the stainless steel cage and had free access to standard pellet and water ad libitum. The rats were exposed to 12-h periods of light and darkness. The temperature and humidity of the room were maintained at 22±2 °C and 65%, respectively. Animal protocols were approved by the Animal ethic Committee of Universiti Kebangsaan Malaysia (FF/2010/Fuad/17 March/298 March 2010-April 2011). The rats were not provided to food 12 h before tests were conducted, but had free access to water.

Rat paw lick test

Twenty four male Sprague Dawley rats were grouped in 4 groups (6 rats for each group). Each group had been given a different injection. The injections were normal saline as a negative control, P1%, P2% and Diprivan® 1% as the positive control, respectively. Injection (100 µl) was given to each rat into the footpad of their hind paws. Then, the number of licking and the total time of the paws being licked was monitored over a period of 15 min. The method was adapted from Lu *et al.* [21].

Sleep recovery test

Eighteen rats were divided to 3 groups and 6 rats for each group (group 1 for P1%, group 2 for P2% and group C for Diprivan®). The test was conducted by giving an intravenous bolus injection of

propofol nanoemulsion for 10 mg/kg body weight of rats on lateral tail vein. Each rat was observed from the beginning after injection. The time when the rats first moved after injection and when the rats become normal (full recovery) were assayed. The methods were adapted from Ravenelle *et al.* [22].

Erythrocyte haemolysis test

The test was performed as reported in the literature [23]. The rat's blood was collected into a test tube containing heparin and centrifuged at 2000g for 5 min. Washed 3 times with 4 volume normal saline. 2% propofol nanoemulsion (2.5 ml) was mixed erythrocytes suspension (0.1 ml) and incubated at 37 °C for 1 hour in a water bath. Then, the sample was centrifuged at 2000g for 5 min. The absorbance from the supernatant was determined by using spectrophotometer UV in wavelength 415 nm to determine % of haemolysis. The percentage of haemolysis was determined by using this equation [24]:

$$\% H = \frac{(Abs\ Test - Abs\ control)}{(Abs\ 100 - Abs\ control)} \times 100\%$$

Where: Abs test= Absorbance of samples

Abs control= Absorbance of the negative control (normal saline)

Abs 100= Absorbance of positive control (distilled water)

Statistical analysis

All the data were analysed using Statistically Package for Social Sciences (SPSS) version 16.0. Data were expressed as means±SD. Data were performed using analysis of variance (ANOVA) test to evaluate the differences between the groups. All experiments were done in triplicate. The differences were considered to be significant at level of *P ≤ 0.05.

RESULTS AND DISCUSSION

Nanoemulsion are one of the dosage form that develop not only for oral route [25, 26], topical usage [27] but also popular for parenteral usage like propofol nanoemulsion. Propofol is one of the anesthetic drug that common usage in the hospital but the pain problem make this drug become not comfortable to use. Free propofol in aqueous phase from the palm oil nanoemulsion was separated from oil phase by using ultracentrifugation methods and measured using HPLC. The chromatograph of propofol in NEMS™ from HPLC result shown in fig. 1 and the results of free propofol content showed in fig. 2. The free propofol concentration of P1% and P2% were 6.20±0.03 µg/ml, 10.55±0.23 µg/ml, respectively. The results were smaller than in Diprivan® 1% (15.02±0.33 µg/ml). Statistical test showed that there was a significant difference between P1% compared with Diprivan® 1% (*P<0.05) whereas P2% showed no significant differences although it showed the smaller free propofol concentration. The results showed that propofol in NEMS™ reduced the intensity of pain because it had less free propofol content compared than Diprivan®1%.

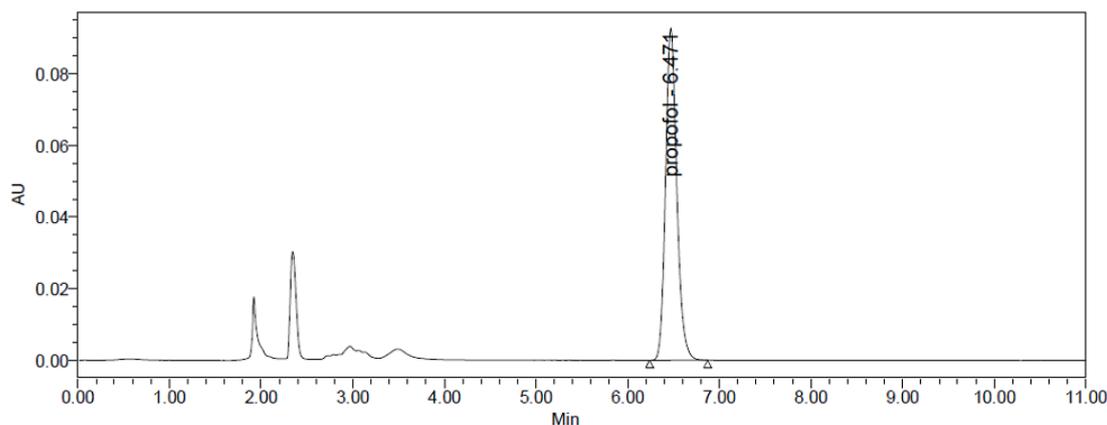


Fig. 1: Chromatograph of propofol in NEMS™

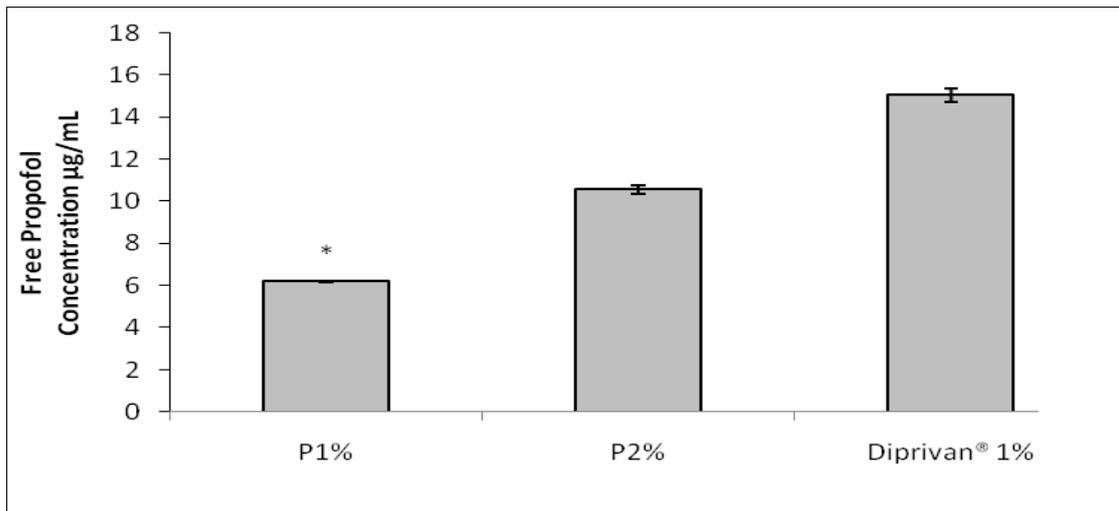


Fig. 2: Free propofol content in P1% and P2% compared with Diprivan® 1% (mean±SD; n=6)

The clear mechanism how propofol induced pain on injection site is still not well understood. One of the possibility is because propofol is one of phenol that can irritate the skin, mucous membrane, and venous intima [28]. The other opinion stated that the activation of the kallikrein-kinin system will induce in pain [29]. Many studies showed that, the intensity of pain related to increase the free propofol concentration in aqueous phase. This is one of the reason why MCT/ICT formulation become one of the solution to decrease this problem [30, 31].

Propofol nanoemulsion in NEMS™ is one of the MCT/ICT formulation for propofol that using palm oil as a basic oil to develop the formulation. From this formulation, hopefully it will reduce the free propofol concentration and it can be used as alternative for propofol carrier in nanoemulsion preparation. Determination of free propofol concentration were performed to assay the free propofol concentration. In this formulation, the concentration of free propofol in NEMS™ is smaller than in Diprivan® from the rat paw lick test, the intensity of pain from propofol in NEMS™ is lower than Diprivan®.

The rat paw lick test was performed to observe if there were a relation between content of free propofol concentration and pain in the animal test. This test was done as *in vivo* test to measure the intensity of pain of propofol nanoemulsion at injection site. The principle of this test was if the intensity of the nanoemulsion increase, so the number of rats licking their paw and the number of time licking also increased [32-34]. As can be seen from table 1, the licking happened in all of the formulations. The results showed that P1% and P2% had less licking compared to Diprivan® 1%. Statistical test showed that there was a significant difference between P1% and Diprivan® 1% (*P<0.05), whereas P2% showed no significant differences. This results showed an appropriateness with free propofol concentration in aqueous phase in case where free propofol concentration of propofol in NEMS™ showed smaller than in Diprivan® therefore it can reduce the pain. In P2%, the free propofol concentration were still quite high because not all of propofol dissolved in oil phase of the nanoemulsion, but it was smaller than Diprivan® nanoemulsion.

Table 1: Results of rat paw-lick test

Formulation	The number of licking (time)	The number of time of licking (min)
Normal saline	2.5±0.7	19.5±6.4
P1%	8.3±1.2*	39.5±6.7*
P2%	9.8±2.0	46.6±12.1
Diprivan® 1%	11.2±1.9	59.5±7.3

(mean±SD; n=6) * P1% (P<0.05) when evaluated with Diprivan® 1%.

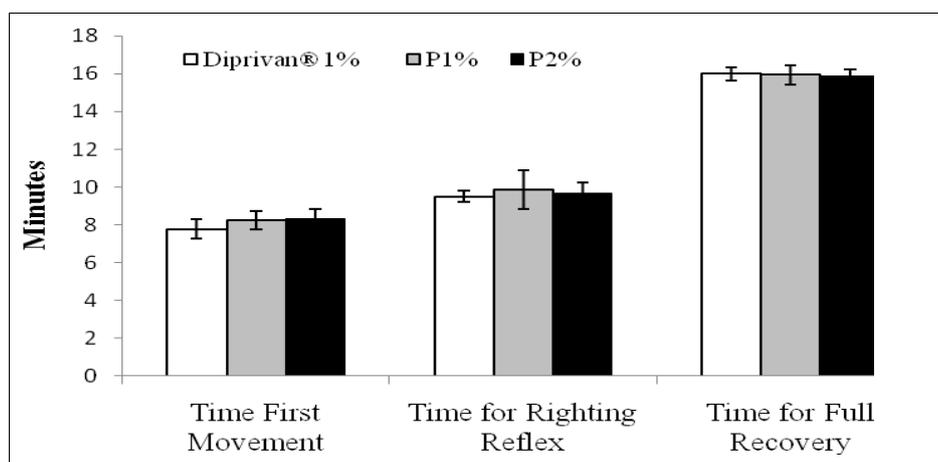


Fig. 3: Profile of sleep test recovery of propofol in NEMS™ compared with diprivan® 1% (mean±SD; n=6)

It was observed from fig. 3, that there were no significant differences ($p>0.05$) between P1%, P2% and Diprivan® 1% as standard. All rats started to sleep not more than 1 min after injection. From this test, the effect of propofol in NEMS™ almost similar with Diprivan® 1% in all parameters test. The rats started to open their eyes after 8 min, try to wake up after 9 min and back in normal condition after 15 min. NEMS™ showed a very good result in pharmacological test. This carrier had similar effect with Diprivan®. It meant that the drug had a good release profile after being injected to the rats. There were no significant differences ($p>0.05$) between all of the formulations.

Haemolysis test

The purpose of this test was to ensure that this formula would give damage or not to the blood [35]. The result showed that distilled water caused 100% hemolysis in blood. Whereas P1%, P2% and Diprivan® 1% were cause a little bit hemolysis in blood, but it was still fulfilled the safety requirement (fig. 4). Parenteral administration of the drug still safety to use if the % haemolysis of the formulation below than 20% [36]. The reason of this phenomena happen is due to the composition of the emulsion like a phospholipid in emulsion would cause interaction with red blood cell [37].

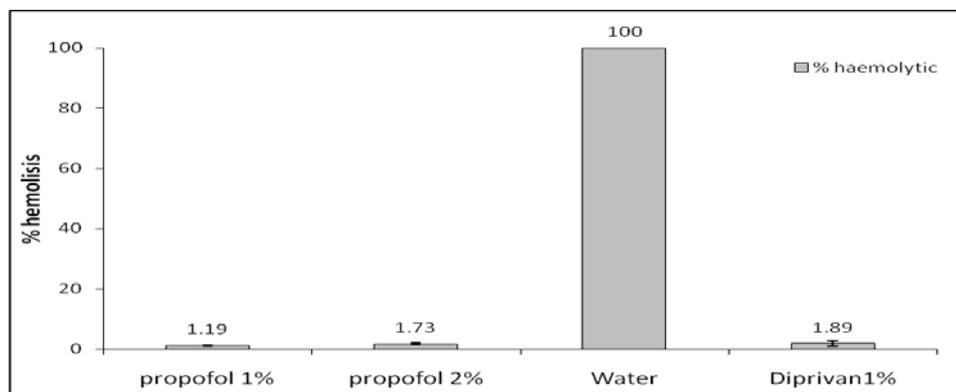


Fig. 4. The amount of red blood cell remaining after hemolysis test (mean±SD, n=6)

CONCLUSION

In summary, palm oil in NEMS™ has proven its potential to produce a propofol nanoemulsion and from the results indicated that palm oil in NEMS™ is promising carrier system for propofol and successfully reduced the free propofol contents and the intensity of pain on injection site in rats.

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AUTHORS CONTRIBUTIONS

All Authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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