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Preparation, Characterization and Anti-Inflammatory Activity of *Swietenia macrophylla* Nanoemulgel

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Abstract

The advances in knowledge about production and stability of dispersed systems enable the development of differentiated vehicles such as nanoemulsions and nanoemulgels, which have been effectively used to increase the bioavailability and improve the stability of the active ingredients. Nowadays there is an intensely usage of natural bioactive materials as medicinal agent in pharmaceutical industries. *Swietenia macrophylla* oil is used due to the bioactivity of different parts of the plant as anti-inflammatory, anti-mutagenicity, anti-tumor. SM oil Nanoemulgels were prepared by incorporating nanoemulsion with hydrogel. First by preparing mixtures of oil, glycerol with sucrose ester (Laurate, Oleate and Palmitate) to produce pre-nanoemulsion using phase inversion technique, then nanoemulsion was produced using self-emulsification technique. After that, hydrogel was added to nanoemulsion to produce nanoemulgel. It was found that 50% oil with sucrose laurate 20% and 30% glycerol was able to produce pre-nanoemulsion, and then it was diluted with water under gentile agitation to produce nanoemulsion with droplets size 114 nm, low size distribution 0.163 and low zeta potential -43.1 mV. The optimal nanoemulsion formulation was mixed with different grades of hydrogel Carbopol 934 and 940 to produce nanoemulgels. It was found that Carbopol showed no influence on the oil droplets size with a range from 113 to 117 nm, size distribution from 0.155 to 0.163 and zeta potential range from -43.4 to -44.6 mV. In addition, it was able to produce a stable nanoemulgel at different temperatures 4°C, 25°C and 40°C when stored for one year and showed priority as thickening agent in relation to texture and rheological properties when compared to Carbopol 934. The anti-inflammatory test using carrageen an induced rat paw edema method for *Swietenia macrophylla* oil was carried and it was found that the inflammation inhibition of SM oil was higher for nanoemulgel compared to oil solution.

Keywords: Nanoemulgel; Nanoemulsion; Hydrogel; *Swietenia macrophylla*; Anti-inflammatory; Carrageenan

Introduction

The technological applications of nanoemulsions have increasingly been used in various applications due to their characteristic properties, small droplet size (in the range 20-200 nm) with high interfacial area, transparent or translucent appearance, high solubilization capacity, low viscosity, and high kinetic stability sedimentation, flocculation, and in some cases, the coalescence [1-3]. In the pharmaceutical field, nanoemulsions have been used as a drug delivery system through various systemic routes mainly: oral, topical and parenteral nutrition [4,5]. The ability to improve the penetration andpermeation of active ingredients through the skin without the need of incorporatepenetration enhancer in the formulation is one of the main advantages of using nanoemulsions topically [5-7]. Different researchers documented an increase in the activity of anti-inflammatory drugs when they are released on skin via a nanoemulsion compared with the conventional emulsion [7-9]. Nanoemulgel which also known as the formation of nanoemulsion-based hydrogel is the addition of nanoemulsion system into hydrogel matrix [10-12]. Usually, hydrogel encounter a limitation of unable to transport lipophilic drugs [12-15]. Therefore, solubilization of lipophillic drug into the oily phase of emulsion which later added into gel base is necessary to enhance limitation of hydrogel besides promoting better stability and drug release [14,16]. This mixture of emulgel has been the attention of many scientists for the development of numerous drugs that function to treat various kind of skin disorders [16,17]. Combining nanoemulsion with hydrogel in forming nanoemulgel has further improved the topical formulation of nanoemulsion. With the gelling system, it promotes better stability of nanoemulsion by reducing the surface and interfacial tension and also enhancing viscosity of the aqueous phase for better administration topically [13,18]. Besides that, drug delivered through nanoemulgel has better adhesion on the surface of the skin and high solubilizing capacity which leads to larger concentration gradient towards the skin, hence influences better skin penetration [14,19,20]. In addition, with the gel based formulation of nanoemulgel, it exhibit upgraded properties of thixotropic, non-greasy, effortlessly spreadable, easily be removed, emollient, not staining, soluble in water, longer shelf life, bio-friendly, translucent and agreeable appearance [13]. Since the formulation is not sticky, hence it eliminate the difficulty of spreading and encourage patient acceptability in administration [15].

Materials and Methods

Materials

Swietenia macrophylla oil was kindly supplied by Nawa Pharma Sdn Bhd (Kuala Lumpur, Malaysia). Sucrose Laurate 1695, Oleate 1570 and Palmitate 1570 were supplied by Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan), and Carbopol 934 and 940 from Acef (Fiorenzuola, Italy). Glycerol was supplied by Sigma-Aldrich (USA).

Methods

Formulation of nanoemulgel: Generally there are several steps

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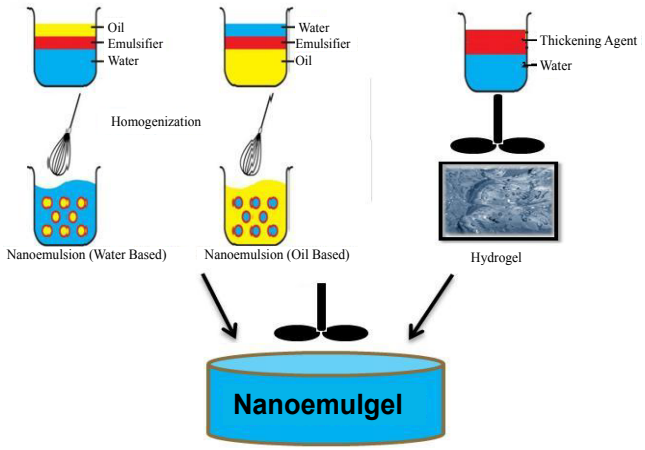

Figure 1: The steps of producing nanoemulgel.

involved in formulation of nanoemulgel, which can be summarized in three steps, first formulation of nanoemulsion. Second formulation of hydrogel using thickening agents, which increase the consistency of any dosage form. Finally nanoemulgel will be produced by the incorporation of nanoemulsion into the gel base with continuous stirring. Nanoemulgel production process presented in figure 1.

Formulation of nanoemulsion: Nanoemulsion was prepared by using both methods phase inversion technique and self-emulsifying system. First a formation of pre-nanoemulsion mixtures required before they could be self-emulsified in water under gentle, to produce nanoemulsion at room temperature. A series of mixtures containing various combinations of *Swietenia macrophylla* (SM) oil as an oil phase with sucrose monoester fatty acid as surfactant and glycerol were prepared to produce pre-nanoemulsion. A Ternary phase diagram was constructed based on three different types of surfactants combination with glycerol and oil at a constant temperature that will produce nanoemulsion. Ternary phase diagram A contained SM oil, sucrose laurate1695 and glycerol while ternary phase diagram B consisted of SM oil, sucrose oleate 1570 and glycerol and Ternary phase diagram C contained combinations of SM oil, sucrose palmitate 1570 and glycerol. The formulations were then used to distinguish the effect of such parameters on the emulsification of the oil. The mixtures were weighed based on ternary diagram using analytical balance (Meller Tolledo). Both of the phases, oil and glycerol, were heated separately at about 75°C ± 5°C by using hot plate, then the surfactant was mixed at 50 rpm speed in a mixing reactor chamber (Ika, Germany) with the hot glycerol until it was completely dissolved, then the previously heated oil phase was added gradually and stirred for about 10 minutes to ensure its totally disappearance. After a proper mixture was attained, the temperature was reduced to room temperature (25°C) to form homogeneous pre-nanoemulsion. The pre-nanoemulsion was then diluted with water and gentle agitated for about 10 minutes to form nanoemulsion. A sample of nanoemulsion was taken, in order to find the efficient region of emulsification. According to the ternary phase diagrams, nanoemulsion (NE) region was marked to indicate the transparent and fine droplets, whereas macro-emulsion (ME) region was marked due to more whitening and isotropic solutions that might contain micelle solutions and coarse emulsion (CE) and was the region of visibly cloudy dispersions even by visual observation. All measurements were performed in triplicates.

Droplet size and zeta potential analysis: The mean droplets size and size distribution for nanoemulsion formulations were measured by laser light scattering using a Master sizer 2000 laser diffractometer (Malvern Instrument, UK), in order to find the efficient region of emulsification. To observe the droplets size and size distribution, 250 µl of the formulation was mixed with 300 ml of distilled water in a 500 ml beaker. A glass rod was used to induce gentle agitation in the mixture. The size distribution reflects the size distribution of particle diameter [21,22]. The measurements were performed in triplicates. All experiments were carried out at room temperature of about 25°C.

Surface charge

The surface charge (zeta potential) of the formulations was analyzed using Zetasizer Nano ZS (Malvern, UK) at room temperature Malvern Nano zetasizer. For this purpose sample of the formulation was diluted with distilled water and then measured by zetasizer. The zeta potential (ZP) is measured in order to characterize the surface charge of particles which gives information about repulsive forces between particles and droplets. Usually ZP indicates good stability of the system if the absolute higher values than +30 mV or lower -30 mV [23].

Nanoemulgel preparation

Nanoemulgels were prepared using 0.5% of different Carbopol grades 934 and 940 and different SM oil concentration (10, 15 and 20%). First Carbopol hydrogels were prepared using Carbopol 934 and 940 as thickening agents, by dispersing Carbopol in purified water an left over night for swelling, then the pH of the hydrogel was adjusted to 5.6 using triethanolamine (TEA) [24]. Then hydrogel matrix was mixed with the optimum nanoemulsion for 10 minutes at 100 rpm until nanoemulgel is formed. Droplets size, size distribution and zeta potential were measured as mentioned earlier.

Physical characterization of nanoemulgel

Nanoemulgel formulations were inspected. Visually for their color, homogeneity, consistency, spreadability, and phase separation. Also the measurement of rheological and texture profiles of the formulations with different concentrations and grades of Carbopol (934 and 940). The pH values of 1% aqueous solutions of the prepared nanoemulgels were measured by a pH meter (CG 820, Schott Gerate GmbH, Hofheim, Germany).

Texture analysis of nanoemulgels

Mechanical properties of nanoemulgels were assessed using a texture analyzer TA-XT2 Plus from Stable Micro System, (Goldalming, UK; Plat 4.2). The back extrusion method was used to measure the texture of nanoemulgels. Data acquisition and mathematical analysis were performed using a computer equipped with the Texture Expert® software version 6 (Texture Technologies Corp. New York).

Rheological properties of nanoemulgel formulations

Rheological characterization is important to evaluate and control the flow properties of semisolid pharmaceutical products to ensure quality and effectiveness of the formulation. Rheological analysis of nanoemulgels to test the oscillation stress sweep was performed using Rheometer Physica MCR 301 (Anton Paar Physica, Austria) with cone-plate geometry sensor with the diameter of the cone being 40 mm and 1° cone angle, operating in the oscillation and static mode. The sample of nanoemulgel to be studied was placed on the plate and left to equilibrate at a controlled temperature (25 ± 0.1°C) for 3 min before bringing the cone down. This was done to ensure the thermal as well as the structural equilibration of all samples. The excess amount of the

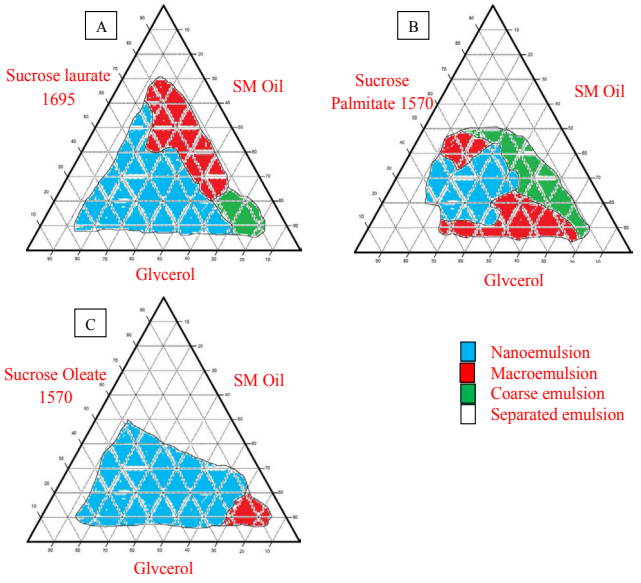

Figure 2: Ternary phase diagrams showing different SM oil emulsifying region for A) system A, B) System B and C) System C.

sample was removed using spatula and tissue papers. The sample was determined in triplicate [25].

Stability of nanoemulgel formulations during storage

Droplet size, size distribution and zeta potential are among the most important characteristics for the evaluation of the stability of emulsion. Therefore, the effects of temperature and storage time were studied on the optimum formulations of nanoemulgel. The droplet size, size distribution and zeta potential were evaluated immediately after the production of the nanoemulgel and also after 1, 2, 4, 6, 8, 10 and 12 months of storage under different temperatures 4, 25 and 40°C.

In vivo anti-inflammatory study

The anti-inflammatory activity was assessed according to the method described by Larson and Lombardino [26] with slight modification. Sprague-Dawley male rats weighting 180-200 g were randomly selected and left for at least 48 hours before the start of the experiment. At all time, rats were handled in accordance with UiTM guidelines for the care of laboratory animals, and the ethical guidelines for the investigations of experimental pain in conscious animals. The animals were kept at room temperature (25 ± 2°C) with 60-70% humidity and 12-hour light/darkness cycle in the Animal Holding Unit. Rats were divided into eight groups of six animals each. These groups were divided according to the formulations administered. The first group served as control (vehicle base), the second group received piroxicam gel (0.5%) and served as a positive control, the third, fourth and fifth groups received SM oil solution of different concentrations 10, 15 and 20% respectively. The sixth, seventh and eighth group received 10, 15 and 20% SM nanoemulgel respectively.

On the test day, 0.1 g of the control material and the formulations were rubbed gently into the right hind paw (hairless leg) until complete disappearance of the applied amount. Five hours later, 0.1 ml of 1% w/v carrageenan suspension was injected into the sub plantar region of the paws of control and treated groups. This was followed by measuring the edema volume via determining paw volume at fix time intervals of 0, 1, 2, 3 and 4 hours using digital paw edema meter (520-R, IITC Life Science - USA)[27]. Mathematically, the degree of swelling was calculated as follow:

$$\% \text{ Change of hind Paw volume} = [(\text{mean } C_n - \text{mean } C_i)/ \text{mean } C_i] \times 100$$

Where C_n is the hind paw volume at 1, 2, 3 and 4 hour intervals following carrageenan injection, and C_i is the initial hind paw volume, before the injection of carrageenan (0 hour) [28,29].

Statistical analysis

Statistical analyses of the anti-inflammatory effects were performed by using one way analysis of variance (ANOVA). A statistically significant difference was accepted at $P<0.05$.

Results and Discussion

Nanoemulgel with small droplet size below 200 nm was achieved first by preparing different combinations of surfactant, glycerol and oil using stirring method to produce nanoemulsion. The optimum nanoemulsion formulation was selected base on the droplet size, size distribution, surfactant concentration and zeta potential. Different grades of Carbopol in various concentrations were used to prepare nanoemulgel. Nanoemulgel was subjected to check their droplet size, size distribution, zeta potential, rhelology and stability study to select the optimum Carbopol concentration and grade. The prepared nanoemulgels were white viscous creamy with a smooth and homogeneous appearance. They were easily spreadable with acceptable bio-adhesion and fair mechanical properties. The pH values of the formulations ranged from 5.5 to 6, which is considered acceptable to avoid the risk of irritation upon application to the skin [30,31].

Influence of surfactants on the formulation of nanoemulsion

The effects of various surfactants were studied for their potential to produce nanoemulsion (Figure 2). Three ternary phase diagrams were constructed to optimize the optimum nanoemulsion formulation. The three different systems showed different behavior in producing nanoemulsion. As a comparison between them, system A showed the largest region of nanoemulsion. It is apparent from figure 2 that the ternary phase diagrams of system A, which comprised of sucrose laurate 1695 as non-ionic surfactant, produces larger region of nanoemulsion compared to other systems due to its good emulsification properties. On the other hand, ternary phase diagrams of system B containing sucrose palmitate 1570 with bad nanoemulsion properties producing small region of nanoemulsion compared to other systems and less emulsification properties compared to sucrose laurate. However, system C containing sucrose oleate 1570 shown has better nanoemulsion region compared to system B with moderate emulsification properties. Sucrose laurate showed the best emulsification properties compared to sucrose palmitate and oleate, which may be due to its good miscibility properties. Same findings were stated by Szuts et al.[32], mentioned that sucrose laurate was good in preparing solid dispersion due to its good miscibility properties compared to sucrose palmitate and sucrose stearate.

Generally, all surfactants produced nanoemulsion formulations with stirring and heat. The capability of producing nanoemulsion was due to the temperature used to dissolve the sucrose ester in the glycerol. The heat treatment of the formulations may lead to changes in the molecular characteristics of the surfactant. Therefore, sucrose ester becomes progressively dehydrated during heating because it is non-ionic surfactant with a hydrophilic head group. For that reason, the surfactant molecules will have changes in the interfacial tension,

packing, and oil/water solubility during heating. Same results were shown in previous studies on non-ionic surfactants that produced micro-emulsions and nanoemulsions formulations by the help of these changes facilitated at higher temperatures [33-36]. In addition, a kinetic energy barrier in the oil-glycerol-surfactant system prevents it from moving from an emulsion to micro-emulsion and nanoemulsion at ambient temperature. But as the temperature was raised this kinetic energy barrier was reduced, which helped in changing from one state to another. Same results were stated by Rao and Mc Clement [36], who used oil-water-surfactant to produce micro-emulsion and nanoemulsion.

Different sucrose esters as non-ionic surfactants were used to study the effect of various HLB values on capability to prepare nanoemulsion formulations. As the degree of sucrose esterification increased and/or the fatty acid chain length increased, the sucrose ester HLB value will be reduced. Oil in water dispersion required HLB value between 9 to 18, so the selection of the optimum HLB value of emulsifying agent depends on its hydrophilicity [37]. System A containing sucrose laurate1695 with high HLB value (HLB 16) was chosen as a non-ionic surfactant, because it produces large region of nanoemulsion formulations with small droplets size, low size distribution and good stability. The ability of sucrose laurate to produce nanoemulsion was due to its good droplets entrapment and stabilization efficacies, which are explained by to the low amounts of di-, tri-, and polylaurates (20%) and higher amount of monolaurate (80%) as well as the lauric acid short chain length. While in system B and system C, sucrose plamitate 1570 (HLB 15) and sucrose oleate 1570 (HLB 15) were used respectively, both surfactants showed nanoemulsion region but it was less compared to system A. that is due to their low HLB value compared with sucrose laurate and less amount of monoesters (70%). Leong and team stated that sucrose laurate 1695 was better than sucrose palmitate 1570, stearate 1570 and oleate 1570 in preparing phytosterolnano dispersions with small particle size below than 100 nm [37]. Therefore, it could be concluded that sucrose laurate as non-ionic surfactant was able to produce nanoemulsion with small droplets size, low size distribution and high stability due to its high HLB value (HLB 16).

Influence of various surfactants ratios on droplets size, size distribution and zeta potential

The droplet size and size distribution were studied for all formulations at the preliminary investigation using Master Sizer Malvern Instrument. Figure 2 showed different nanoemulsion regions when different surfactants have been used. Their droplets size and size distributions were varied. System A containing sucrose laurate led to the formation of oil droplets with the smallest size and size distribution compared to other systems B and C. For system A the formulation which showed the smallest droplets size was 114 nm with size distribution 0.163. But system B containing sucrose palmitate showed significant higher droplets size 365 nm and 0.302 size distribution compared to system A. While system C having sucrose oleate showed better results compared with system B but still not as small as system A. It droplets size were 221 nm with 0.291 size distribution.

Table 1showed the chosen points of nanoemulsion formulations from system A, based on droplets size, which should be less than 200 nm. Sucrose laurate led to production of nanoemulsion formulations with various oil concentrations between 20 to 50%. From the pseudo-ternary phase diagram it can be observed that when the oil concentration in the formulations increased from 20% to 50%, their droplets size was decreased and form more stable formulations. This attributed to the fact that sucrose ester will be bounded to the surfaces of the oil droplets when the viscosity of the formulations were increased and therefore will be less available to participate in the formation of a phase. Similar findings were reported by Murakami et al. [38]and Rao and Mc Clement [36], they stated that the viscosity has an influence in forming stable colloidal dispersion formulation with small droplets size by using sucrose ester as non-ionic surfactant. But as the oil concentration goes up more than 50% the droplets size were increased and separation happened due to less amount of surfactant to form colloidal dispersion and stabilize the system.

In general, the formulations B, D and F that containing 50% oil concentration showed smaller droplets size with good size distribution compared to formulations A, C and E that containing 36% oil concentrations. Also in a comparison between the formulations with 50% oil we found that formulation B contain 15% surfactant has larger droplet size and size distribution compared to formulations D with 20% surfactant and F with 25% surfactant. The high size distribution for formulation B which was higher than 0.3 makes it unstable when compared to other formulations which have size distribution below 0.3. In the other hand, both formulation D and F showed almost similar droplet size and size distribution. However, formulation D will be better than F because it has less amount of surfactant. Therefore, we can conclude that the combinations which contain 20% sucrose laureate 1695 and 50% oil will produce a stable nanoemulsion with small droplet size and size distribution.

In addition, all formulations showed high value of zeta potential charge above -30 mV, which indicates that nanoemulsion formulations are stable [23,39]. Formulation B showed slightly low zeta potential charge which indicates that this formulation might be unstable over storage because this formulation has low amount of surfactant which is not enough to make it stabilized. In summary, formulations containing 50% oil showed smaller droplets size and zeta potential compared to formulations containing 36% oil, as well in a comparison between the formulation containing 50% oil, formulation D with the optimum amount of surfactant which shows the smallest droplets size, size distribution and the best zeta potential. Also it is known that large amount of surfactants cause skin irritation, therefore, formulation D will be the optimum formulation compared to other nanoemulsion formulations.

Formulation	Laurate (%)	Glycerol (%)	SM oil (%)	droplet size (nm) ± SD	Size distribution ± SD	Zeta Potential (mV) ± SD
A	19.2	44.8	36.0	183 ± 1.3	0.297 ± 0.001	-33.1 ± 0.9
B	15.0	35.0	50.0	174 ± 1.1	0.554 ± 0.008	-27.4 ± 1.6
C	25.6	38.4	36.0	132 ± 1.4	0.299 ± 0.003	-36.4 ± 1.3
D	20.0	30.0	50.0	114 ± 0.7	0.163 ± 0.006	-43.1 ± 0.8
E	32.0	32.0	36.0	129 ± 1.1	0.262 ± 0.002	-37.6 ± 1.2
F	25.0	25.0	50.0	120 ± 1.7	0.208 ± 0.004	-39.2 ± 1.7

All data are presented as mean ± SD, (n= 3).

Table 1: Chosen point of nanoemulsion formulations from system A.

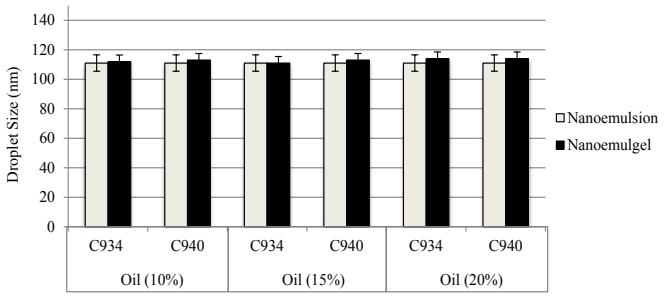

Figure 3: Droplets size measurement of nanoemulgel containing different concentrations of SM oil and Carbopol grades.

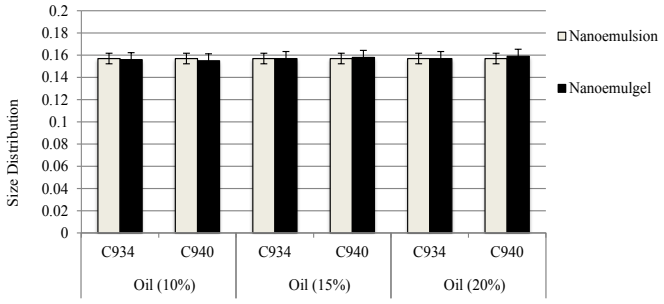

Figure 4: Size distribution measurement of nanoemulgel containing different concentrations SM oil and Carbopol grades.

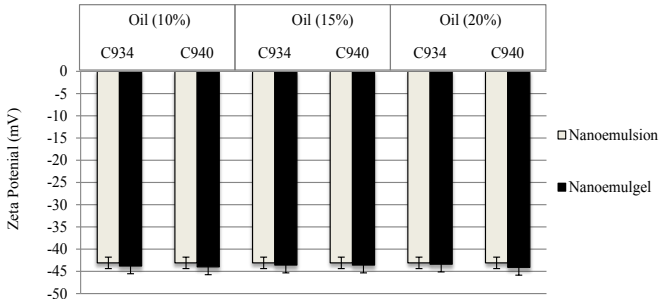

Figure 5: Zeta potential measurement of nanoemulgel containing different concentrations of SM oil and Carbopol grades.

Nanoemulgel formulations

Nanoemulgels containing SM oil were prepared using two different types of Carbopol (934 and 940) and different oil concentrations 10, 15, 20%. The ling and swelling properties of Carbopol contributed to its use as thickening agents [40]. The optimum formulation nanoemulsion which contain sucrose laureate (20%), glycerol (30%) and oil (50%) was mixed with water and then the Carbopol solution was added with continuous mixing to form the nanoemulgel. Nanoemulgel formulations were subjected to study for their droplets size, size distribution, zeta potential and also their rheological behaviors. Stability study for the period of one year at three different storage temperatures 4, 25 and 40°C was conducted for the optimum formulations and *in-vitro* permeation of the optimum formulation through rate skin was studied.

Influence of various Carbopol concentrations on droplets size, size distribution and zeta potential: The mean droplet size, size distribution and zeta potential of different nanoemulgel formulations were measured and compared with the results of the optimum nanoemulsion formulation. Figure 3 shows the comparison of initial nanoemulsion with the mean droplets size of nanoemulgel formulations containing different grades and SM oil concentration.

The mean droplets size of SM oil nanoemulgel formulations were ranged from 113 to 117 nm. It was noticed that after adding Carbopol as thickening agent, there were no significant changes in the mean droplets size. Same results were reported by Yilmaz and Bolchert [24], they found that the mean droplets size of nanoemulgel prepared by the addition of Carbopol 940 as thickener agent, to nanoemulsion formation was not significantly changed compared to nanoemulsion formulation. But only slight increment in the mean droplets size, which were due to the increment in the viscosity by adding Carbopol thus resulting in enlargement of the droplets size. The same findings were stated by different authors, who stated that adding polymer resulted in the increase of the viscosity of the medium that resulted from a high degree of cross-linking. Hence, larger will be the size of droplets [41,42].

The size distributions for nanoemulgel formulations (Figure 4) were ranged from 0.155 to 0.163. The figure showed no significant change in the size distributions. In addition, nanoemulgel formulations showed high zeta potential above than 40 mV, which indicates that SM oil nanoemulgel formulations have good stability. This has been approved by Jeong and colleagues, who stated that emulsions with high negative or positive zeta potential values gain their stability through increasing electrostatic repulsion between the emulsion droplet surfaces and prevention of droplet coalescence [43]. Figure 5 showed the zeta potential values for SM oil nanoemulgel formulations, the figure showed no significant changes in zeta potential, which range -43.4 to -44.6 mV, the little difference in zeta potential values were due to the addition of Carbopol. This indicated that a slight increase in the zeta potential values with the addition of Carbopol as thickening agent which influences the surface charge of the droplets.

Texture characteristics of nanoemulgels: There were three different parameters evaluated in order to characterize the texture of nanoemulgels, namely firmness, consistency and adhesiveness. Such parameters have been used in the development of cosmetic and pharmaceutical semi-solid systems to provide related information [44,45]. Hardness of a semisolid product is always represented by the firmness and it is related to the high viscosity and consistency of the product. The maximum force required to break the product is the measurement parameter of firmness; the higher the value, the firmer the sample. Consistency is a common textural property of semi-solid products. It is most often measured using the back extrusion rig. Cohesiveness is defined as the work needed to overcome the attractive forces between molecules within the sample. It is the negative force area and represents the work required to overcome the attractive forces between molecules in the sample [45].

The texture analysis by using back extrusion method generated

Carbopol	SM Oil (%)	Firmness (g)	Consistency (g.sec)	Cohesiveness (g)
940	10	1357.02	1423.89	-390.1
	15	1550.17	1528.88	-385.53
	20	2210.19	2130.1	-679.76
934	10	320.7	342.14	-258.41
	15	378.14	483.7	-262.52
	20	718.64	910.22	-324.24

Table 2: Firmness, consistency and cohesiveness for SM nanoemulgel containing Carbopol 934 and 940.

three parameters, namely firmness, consistency and cohesiveness. Both Carbopols 934 and 940 in nanoemulgel gave similar responses on the firmness values by the increase in the oil concentration as shown in the table 2. The increase in the oil concentration did give significant change on the firmness value. The firmness response of Carbopols 934 and 940 was found to be significantly different, with superiority of Carbopol 940 over Carbopol 934. The response on the consistency behavior by interaction of oil concentration and type of Carbopol, showed significantly different. The effect of the increment in oil concentration on consistency response was similar to response of firmness as can be seen in table 2, since the firmness value depended on the consistency of nanoemulgel. The nanoemulgel cohesiveness response of Carbopols 934 and 940 showed similar response to firmness and consistency. Which shown that cohesiveness increases significantly by the increase in oil concentration. In general, it would be said that nanoemulgel containing Carbopol 940 had better texture over 934 which indicates that Carbopol 940 have superiority as thickening agent for nanoemulgel over Carbopol 934.

Influence of Carbopol grades and oil concentrations on the rheological properties of nanoemulgel: The rheological analysis by using the oscillation frequency sweep methods generated three parameters, namely storage modulus (G`) (Pa), loss modulus (G``) (pa) and complex viscosity (Eta) (Pa.s). SM oil nanoemulgel of Carbopol 934 and 940 (Figures 6 and 7) showed a similarity of profile, both Carbopols showed an increase in the storage modulus, but it was higher in Carbopol 940 compared to 934. In addition, both Carbopols

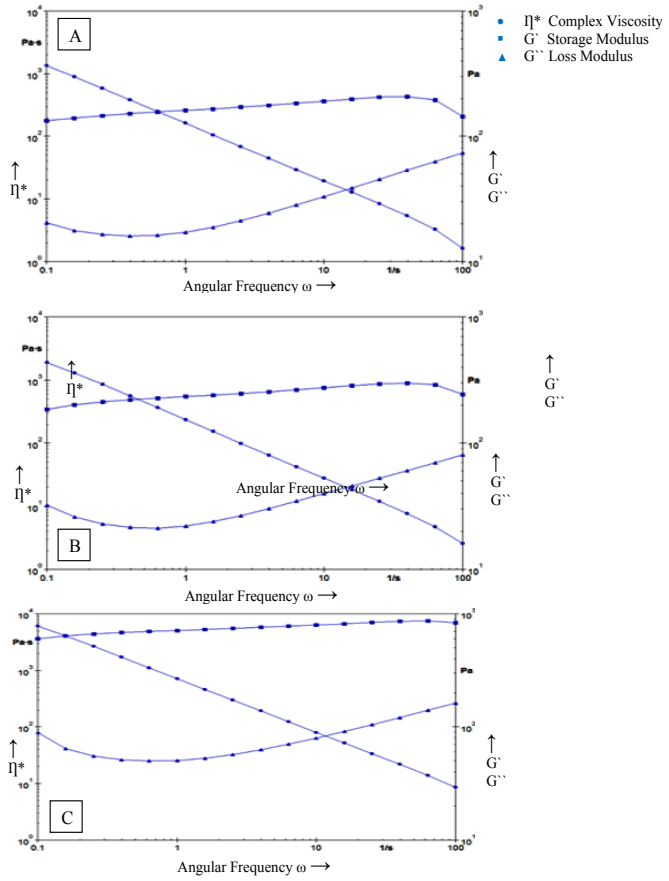

Figure 6: Rheogram of Carbopol 934 nanoemulgel containing A) 10% SM oil, B) 15% SM oil and C) 20% SM oil.

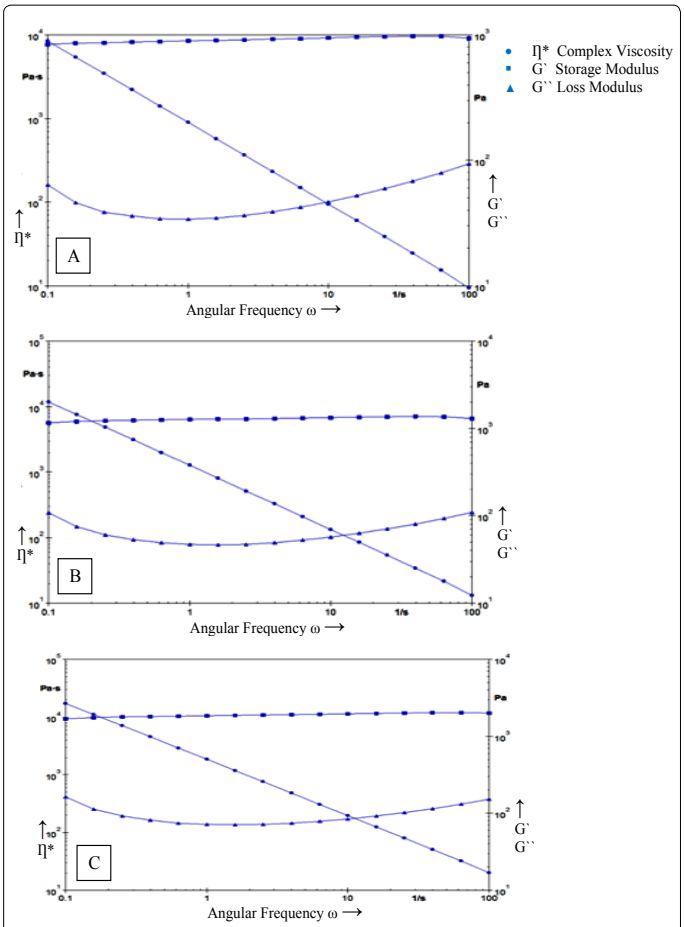

Figure 7: Rheogram of Carbopol 940 nanoemulgel containing A) 10% SM oil, B) 15% SM oil and C) 20% SM oil.

nanoemulgels have shown an increase in the storage modulus and loss modulus when the oil concentration was increased. The loss modulus increased to the maximum at almost equal volume for both carbopol 934 and 940 nanoemulgels. The complex viscosity response of all formulations was higher for Carbopol 940 nanoemulgel when compared with Carbopol 934 nanoemulgel. However, the profile interaction with different oil concentrations for both Carbopols nanoemulgels was similar, which was shown that the increased in oil concentration increased response of the complex viscosity. The analysis of oscillation frequency sweep parameters of Carbopols nanoemulgels for the SM oil formulations was shown interesting results. The parameter responses showed a significant different for storage modulus, loss modulus and complex viscosity, this is showed the superiority of Carbopol 940 as viscosity modifier in nanoemulgels compared to Carbopol 934. For the selection of nanoemulgel formulation for cosmetic application, criteria of a good rheological character are very important to ensure product stability, active ingredient permeation rate and product acceptability by consumer. Therefore, Carbopol 940 has been chosen and a viscosity modifier for SM oil nanoemulgel.

Influence of different storage temperatures on the droplets size, size distribution and zeta potential: Droplet size, size distribution and zeta potential are the most important physical characteristics of a nanoemulsion and nanoemulgel used for topical preparations to evaluate their activity effect, bioavailability and mostly to determine their stability against gravitational separation and flocculation [47]. The

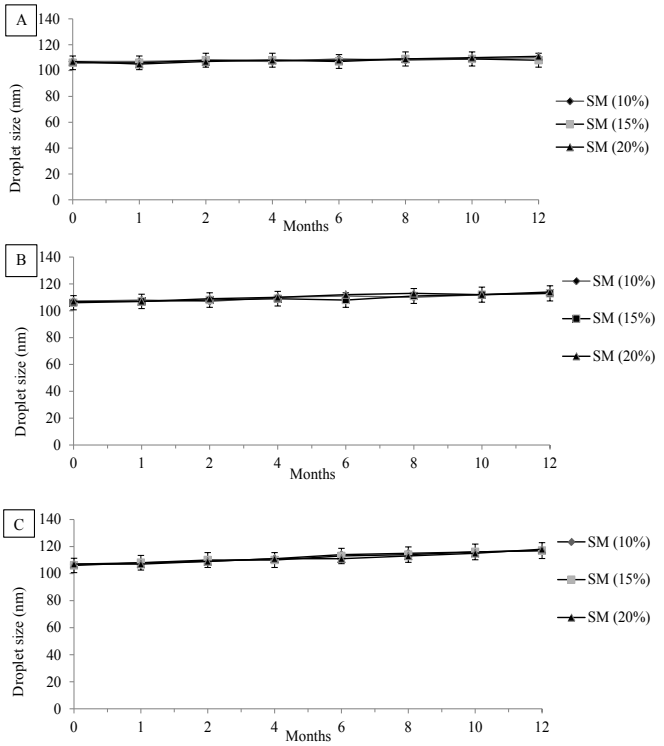

Figure 8: Droplets size measurement of SM oil nanoemulgels subjected to different storage temperatures (A) 4°C (B) 25°C and (C) 40°C, after one year of storage.

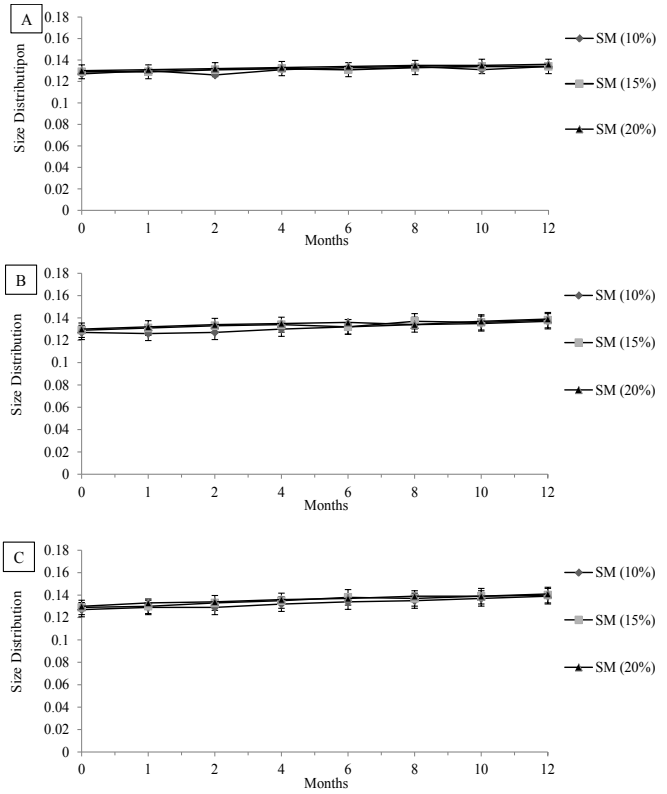

Figure 9: Size distribution measurement of SM oil nanoemulgels subjected to different storage temperatures (A) 4°C (B) 25°C and (C) 40°C, after one year of storage.

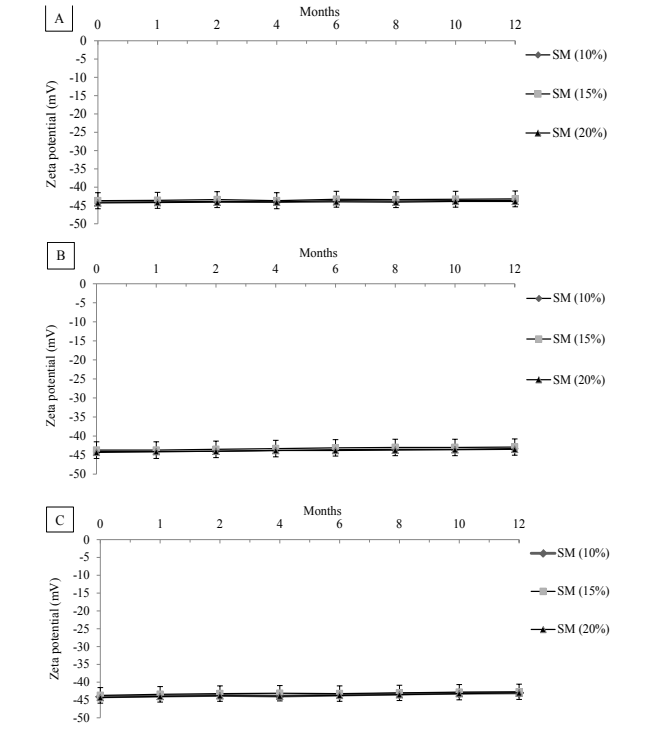

Figure 10: Zeta potential measurement of SM oil nanoemulgels subjected to different storage temperatures (A) 4°C (B) 25°C and (C) 40°C, after one year of storage.

rheological modifiers like Carbopol have a great effect on the droplets size and on the stability by acting as an emulsifier and stabilizer [47].

The droplets size, size distribution and zeta potential parameters were evaluated immediately after the production of nanoemulgel and over one year (1, 2, 4, 6, 8, 10 and 12 months) of storage at three different temperatures (4, 25 and 40°C). It can be observed that there are no significant changes in the droplets size at 4°C, 25°C and 40°C stored for one year (Figure 8). In addition, there were no significant changes occurred to size distribution for SM oil at the storage for one year (Figure 9). As well, zeta potential showed no significant changes when stored at different temperature over a period of one year (Figure 10).

Therefore, it can be concluded from the results presented in these figures that nanoemulgel formulations containing different concentrations of oil having 0.5% Carbopol 940 as thickening agent werestable over a period of one year at different storage temperatures.

The good stability of the nanoemulgel formulations may be attributed to the good stability of the initial nanoemulsion used in the preparation of nanoemulgels also mainly due to the addition of Carbopol 940 as thickening agent. Same findings were reported by Mohamed [48], who reported that the use of thickening agents will help in stabilizing nano-scale emulsions containing Iboprofen. Also Abdullah et al. [49] stated that the stability of nanoemulsion containing Carbopol 940 to modify its viscosity, was ascribed to the type of surfactant used and the Carbopol 940 as a rheology modifier of the nanoemulsion.

Anti-inflammatory activity of topical preparations of *Swietenia macrophylla* oil: The anti-inflammatory drug penetrates the skin slowly when applied topically and passes to the systemic circulation in small

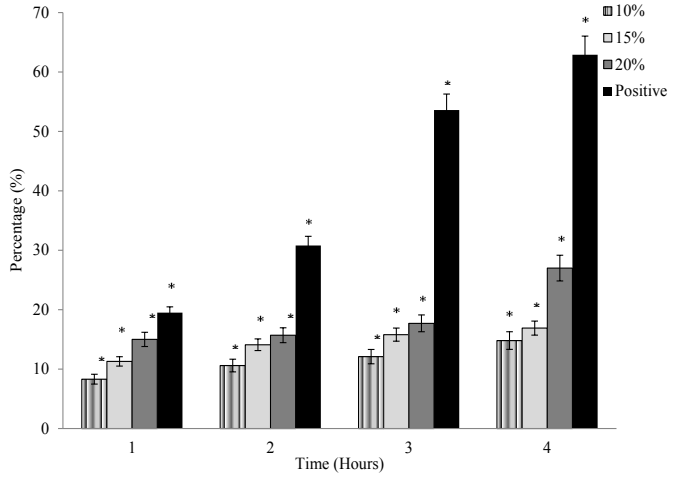

Figure 11: Percentage of topical anti-inflammatory inhibition of *Swietenia macrophylla* oil solutions used in different concentrations. (*indicate significant when P < 0.05).

Paw volume (ml)					
Group	0 (hr)	1 (hr)	2 (hrs)	3 (hrs)	4 (hrs)
10%	0	0.127 ± 0.015 (8.3 %)	0.177 ± 0.020 (10.6 %)	0.233 ± 0.033 (12.1 %)	0.202 ± 0.026 (14.8 %)
15%	0	0.118 ± 0.018 (11.3 %)	0.17 ± 0.028 (14.1 %)	0.223 ± 0.032 (15.8 %)	0.197 ± 0.036 (16.9 %)
20%	0	0.113 ± 0.016 (15 %)	0.167 ± 0.023 (15.7 %)	0.218 ± 0.020 (17.7 %)	0.173 ± 0.026 (27 %)
Positive	0	0.107 ± 0.029 (19.5 %)	0.137 ± 0.028 (30.8 %)	0.123 ± 0.031 (53.6 %)	0.088 ± 0.044 (62.9 %)
Negative	0	0.133 ± 0.023 (-)	0.198 ± 0.020 (-)	0.265 ± 0.023 (-)	0.237 ± 0.035 (-)

Value is in mean ± SD. Number of animal each group is (n=6).

Table 3: Change in rat hind paw volume and percentage of inhibition of topically applied *Swietenia macrophylla* oil solution at different oil concentrations.

quantities. Generally, the bioavailability of anti-inflammatory drugs is 5 to 15% less when compared to the equivalent oral administration [8]. The extent of penetration level of the anti-inflammatory drugs through different skin is rendered by the skin composition. Therefore, the purpose for the enhancement of topical delivery drugs is to target underlying tissues [8,50].

Swietenia macrophylla oil solutions in different concentrations (10, 15 and 20%) were tested for their anti-inflammatory activity via carrageenan-induced paw edema test. It was by topical application of the oil. Different concentrations of *Swietenia macrophylla* oil (Figure 11) showed a weak anti-inflammatory activity when compared with the positive control (piroxicam gel 0.5%). The highest percentage of inhibition was 27% in the 20% concentration *Swietenia macrophylla* oil, while it was 14.8% and 16.9% inhibition activities in 10% and 15% oil concentration respectively (Table 3). In the other hand, the positive control (Piroxicam gel 0.5%) showed significantly higher inhibition activities, 62.9% when compared with *Swietenia macrophylla* oil. This indicates that the *Swietenia macrophylla* oil in the form of oil solution has low topical anti-inflammatory activities when compared with the positive control.

The topical application of Nanoemulgel containing *Swietenia macrophylla* oil to rats produced significantly high anti-inflammatory activity at different concentrations (Figure 12). The percentage of inhibition of nanoemulgel containing different oil concentrations, exhibit significant anti-inflammatory activity when compared with the negative controls. The maximum inhibition was 69.6 % for the 20% concentration of the oil nanoemulgel. The nanoemulgel with 15% and 20% oil concentrations, showed significantly higher inhibition activities (67.1% and 69.6%) when compared with the positive controls (62.9%). While results were almost similar in the positive controls compared with 10% nanoemulgel with (61.2% inhibition) (Table 4).

The above results showed variation in the topical anti-inflammatory activities between oil solution and nanoemulgel of *Swietenia macrophylla* oil used at the same concentration. It was observed that the oil solution exhibit low anti-inflammatory activities when compared with the positive control, while nanoemulgel showed higher or similar activities when compared with the positive control. The variation in results could be due to the nano-size oil droplets, and could be attributed to the high penetration of the nanoemulgel oil droplets through rat skin, while the penetration is very low in oil solution.

Conclusion

In conclusion, nanoemulgel was prepared first by formulating nanoemulsion then hydrogel containing Carbopol was added to form nanoemulgel. Nanoemulsion was prepared using sucrose ester as surfactant blended with glycerol and oil to form nanoemulsion. The nanoemulsion properties were affected by the nature and the concentration of sucrose ester surfactant. Sucrose laureate surfactant

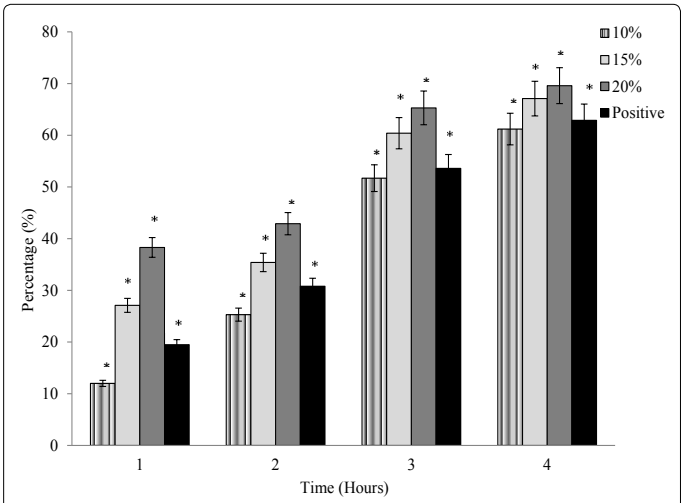

Figure 12: Percentage of topical anti-inflammatory inhibition of *Swietenia macrophylla* oil nanoemulgels at different oil concentrations. (*indicate significant when P < 0.05).

Paw volume (ml)					
Group	0 (hr)	1 (hr)	2 (hrs)	3 (hrs)	4(hrs)
10%	0	0.117 ± 0.019 (12 %)	0.148 ± 0.015 (25.3 %)	0.128 ± 0.017 (51.7 %)	0.092 ± 0.020 (61.2 %)
15%	0	0.01 ± 0.022 (27.1 %)	0.127 ± 0.024 (35.4 %)	0.105 ± 0.015 (60.4 %)	0.078 ± 0.015 (67.1 %)
20%	0	0.082 ± 0.015 (38.3 %)	0.113 ± 0.024 (42.9 %)	0.092 ± 0.026 (65.3 %)	0.071 ± 0.013 (69.6 %)
Positive	0	0.107 ± 0.029 (19.5 %)	0.137 ± 0.028 (30.8 %)	0.123 ± 0.031 (53.6 %)	0.088 ± 0.044 (62.9 %)
Negative	0	0.133 ± 0.023 (-)	0.198 ± 0.020 (-)	0.265 ± 0 .023 (-)	0.237 ± 0.035 (-)

Value is in mean ± SD. Number of animal each group is (n=6).

Table 4: Change in rat hind paw volume and percentage of inhibition for nanoemulgel containing *Swietenia macrophylla* oil used at different concentrations.

showed better nanoemulsion properties with small droplets size, low size distribution and high negative zeta potential value compared to sucrose oleate and palmitate. Nanoemulgels were sensitive to the grade of Carbopol. Carbopol 940 at 0.5% showed priority as thickening agent over 934 in relation to texture and rheological properties of nanoemulgel. In general the droplets size, size distribution and zeta potential of the nanoemulsion were not influenced with the addition of Carbopol. Nanoemulgels were stable under different storage temperatures for one year, the oil droplets size, size distribution and zeta potential were not influenced upon the storage. Finally, *Swietenia macrophylla* oil solution showed low anti-inflammatory activity when compared with the positive controls, but the anti-inflammatory activity of SM oil were improved when the oil was applied in the form of nanoemulgels. *Swietenia macrophylla* oil nanoemulgel has higher anti-inflammatory activity when compared with the positive controls.

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