Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):843-849

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Niosomal transdermal gel formulation of curcumin having anti-inflammatory effect in experimental rat models

Latifah Rahman[1], Arisanty[2] and Marianti A. Manggau[3]

[1]Department of Pharmaceutical and Technology, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia
[2]Department of Pharmacy, Politeknik Kesehatan Makassar, Makassar, South Sulawesi, Indonesia
[3]Department of Biopharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia

---

ABSTRACT

Curcumin is anisolated active compound of the spice turmeric, has a long history as a medicinal herbal for a variety of diseases. Transdermal drug delivery like niosomal gel as a transdermal formulation has been recognized as an alternative route to oral delivery. In this study, curcumin was formulated into niosome; characterized by light microscopy and their drug entrapment efficiency. Curcumin-niosomes were formulated again into gel: ex vivo drug permeation and in vivo anti-inflammatory effect were evaluated. The result showed that Formula B (span 60: cholesterol = 7:3 in mmol ratio) has the best characters: morphology surface (multilamellar vesicles), particle size (1-5 µm) and entrapment efficiency (61.22±0.004%). Formula B that formulated onto gel has an anti-inflammatory effect on peptone-induced inflammation. In conclusion, our data show that curcumin successfully formulated as niosomal transdermal gel and are possible candidates as anti-inflammation therapies.

Keywords: curcumin, niosomal gel and anti-inflammation

INTRODUCTION

Curcumin is a yellow pigment from *Curcuma longa*, the main active compound in spice turmeric[1, 2]. Curcumin commonly used as a spice and food-coloring agent[3]. Nowadays, curcumin used in clinical because its powerful medicinal properties. Pharmacological properties of curcumin are: against amyloid-β-induced cell damage on Alzheimer's disease [4]; inhibited rat colorectal carcinogenesis by activating PPAR-γ[5]; inhibits growth of prostate carcinoma via miR-208-mediated CDKN1A activation[6]; anti-leukemia effects of imatinib by down-regulation of the AKT/mTOR pathway and BCR/ABL gene expression in Ph+ acute lymphoblastic leukemia[7]; antioxidant capacity in vivo study[8]; and a stronger antioxidant [9, 10].

In Ayurvedic, curcuminoids has a long history and used as a treatment for inflammatory conditions[11]. Inflammation is the body's attempt at self-protection to remove harmful stimuli including: damaged cells, irritants, or pathogens[12] but excessive inflammation can cause some disease [13]. Antioxidant ability of curcumin is associated to its anti-inflammatory effect [14, 15]. Curcumin inhibit the mRNA and protein expression of COX-2, but not COX-1 on HT-29 cells[16]. Despite inhibited COX-2, curcumin also inhibits lipoxygenase by binding to its central cavity[17].

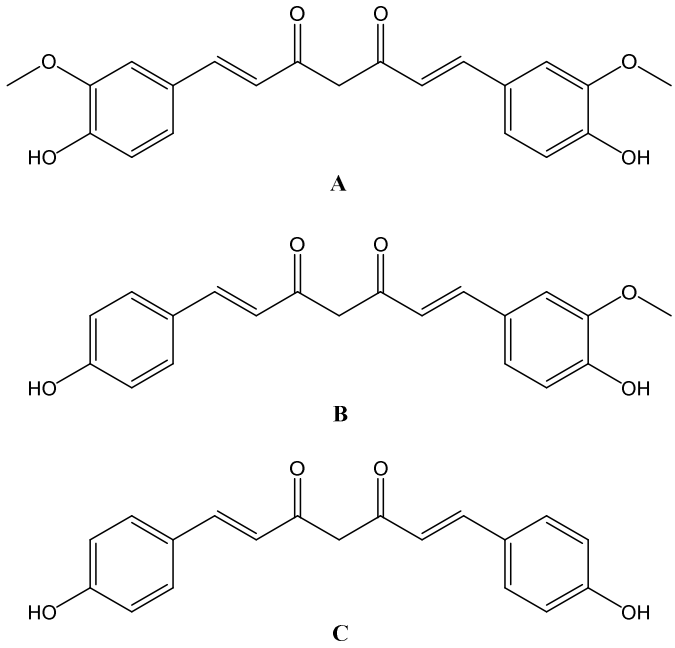

Figure 1.Turmeric constituents include the three kind of curcuminoids: (a) curcumin, (b) demethoxycurcumin, and (c) bisdemethoxycurcumin.

In recent years, niosome is alternative as carriers to deliver active ingredients to the skin or throughthe stratum corneum. Niosome is microscopic non-ionic surfactant vesicles formed by the self-assembly of non-ionic surfactant[18]. Have higher physicochemical stability, more penetrating capability without significant toxicity in the skin after topical application [19]. As an alternative treatment forinflammation and to improve compliance and convenience for application, the study is intended to determine anti-inflammation effect of curcumin niosomal gel *in vivo*.

EXPERIMENTAL SECTION

Materials

Curcumin isolated from *Curcuma longa* L. (No catalogue 239802), cholesterol, carbopol, sorbitan monopalmitate (span 20, 60 and 80), propylene glycol, glycerol, methyl paraben, diethyl ether, phosphate buffer pH 6.8 and peptone were purchased from E Merck (Indonesia). Diclofenac gel® was purchased from local drug store.

Preparation of curcumin-niosomes

Curcumin-niosomes were prepared by reserve phase evaporation with various concentration of span and cholesterol (Table 1). Span and cholesterol solutions in diethyl ether were added curcumin in sonication tube. This solution was added phosphate buffer pH 6.8 and sonicated for 1 min. The mixture was transferred to a 100mL round bottom flask andevaporated using a rotary evaporator at 60^{O}C until a thin film was formed. The dried lipid film was subsequently hydrated with phosphate buffer and the mixture was shaken in a water bath for 2 h until niosome vesicles were formed. The supernatant was decantedand the niosomes were collected for further characterization.

Table 1. Composition of curcumin niosomal formulation containing surfactant (Span 20, 60 and 80) and cholesterol with some ratio

Formulation	Surfactant		Curcumin (mg)
	Type of span	Span : cholesterol ratio (mmol)	
A	20	7 : 3	20
B	60	7 : 3	20
C	80	7 : 3	20
D	20	7 : 2	20
E	60	7 : 2	20
F	80	7 : 2	20
G	20	8 : 3	20
H	60	8 : 3	20
I	80	8 : 3	20

Morphology and formation of curcumin-niosomes
Niosome vesicle morphology was confirmed using optical microscope. The curcumin-niosomes were resuspended in aqueous and transferred onto a glass substrate. The specimenswere then dehydrated with ethanol and dried with a critical point.

Determination of entrapment efficiency (EE)
Niosome ability vesicle to entrap curcumin was determined by spectrophotometry UV-Vis. The percentage of entrapment efficiency was calculated as:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Entrapped curcumin}}{\text{total of curcumin}} \text{ x } 100\%$$

Preparation of Curcumin niosomal gel
Curcumin niosomal gel was made in carbopol gel. Two g carbopol were suspended in aqueous, added curcumin niosome and mixed using magnetic stirrer. The mixture was added 1% of propylene glycol, 15% of glycerol and 0.18% of methyl paraben (methyl paraben in hot aqueous). The gel was packed in wide mouth plastic jars, kept in cool place for further study.

Ex vivo skin permeation study
Ex vivo skin permeation studies were performed onFranz diffusion cell with an effective diffusional area of2.52 cmand 20 mL of receiver chamber capacity using human penis skin as permeation membrane. Human penis skin samples were obtained from patients undergoing circumcisionsurgery. Human penis skin was prepared as described in a previous work[20].

Animal preparation
The male Wistar albino rats weighing 180–200 g were used for the study. The rats were group-housed in cages with five animals per cage, maintained under standard laboratory conditions with dark-light cycle. All rats were allowed access to standard pellet diet and water *ad libitum*. water ad libitum. All the experiments were carried out using three group and each group containing three animals.

Anti-inflammation effect
Acute inflammation was induced by injecting 0.1 mL of peptone (1% in saline) locally into the plantar aponeurosis of the right hind paw of the rats. Group I served as negative control, received vehicle (carbopol gel without contain curcumin niosome). Group II received 200 mg/cm^2 curcumin niosomal gel and group III as positive control, received diclofenac gel® (2 mg/cm^2). The curcumin niosomal gel and diclofenac gel® were administered in tropical usage on plantar aponeurosis of rat. The edema volume up to the ankle joint was measured using a Plethysmometerat 0 h (just before carrageenan injection) and then at 1, 2, 3, 4 and 5 h.

Anti-inflammation effect was calculated as percentage of edema volume inhibition. The percentage of edema inhibition calculated as:

$$\text{Inhibition of edema volume (\%)} = \frac{V_{NC-V_T}}{V_{NC}} \text{ x } 100\%$$

Note:
V_{NC}=mean increase in paw volume in negative control groups
V_T=mean increase in paw volume in treated groups

STATISTICAL ANALYSIS
Data were expressed as means±SD. Statistical differences between the treatment and the respective control groups were evaluated by one-way ANOVA followed by Tukey-Kramer post hoc test, $p>0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Characterization of curcumin niosomes
The morphology of curcumin niosomes was investigated using optical microscopy. Use of span 20, 60 and 80 produced niosomes with multilamellar surface layer.

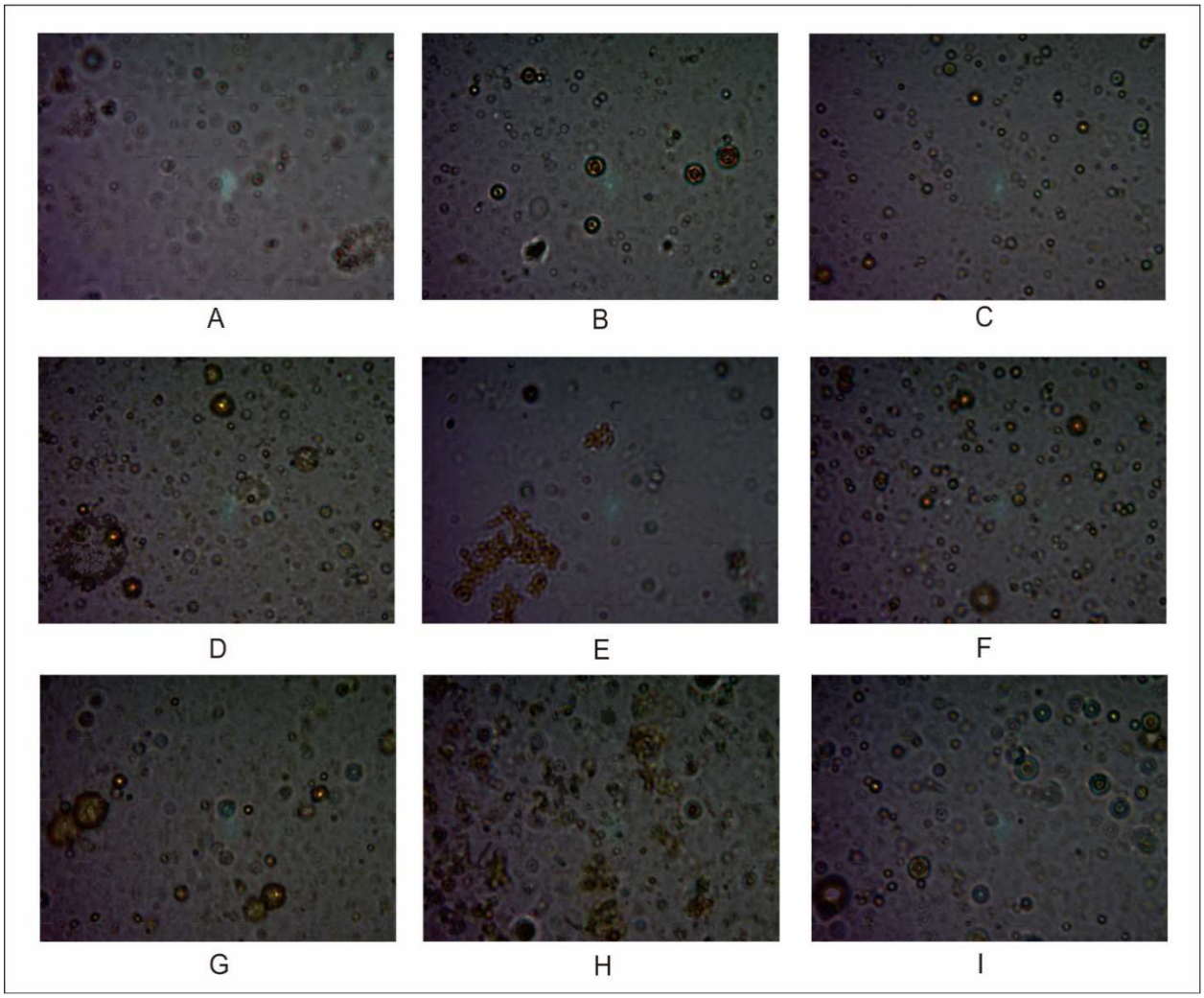

Figure 2. Morphology of curcumin niosome prepared using span 40, span 60 and span 80 as non-ionic surfactants

The size and shape of the niosome vesicle is strongly influenced byvalue of hydrophilic-lipophilic balance (HLB) that surfactant used[21]. HLB of a surfactant is a measure of the degree to which it is hydrophilic or lipophilic which can be determined by calculating values for the different regions of the molecule.High value of HLB will increase the vesicle size of niosome, the surface free energy is going down on more hydrophobic surfactant[22]. Lipophilic drug like curcumin will produce a big size of vesicle niosome 1-5 µm (Figure 2). Vesicles size from 150 nm to 2 µm or higher can induce macrophages phagocytosis in plasma, no effective for oral or injection administration but still allowed in tropical usage [23].

Table 2. Entrapment efficiency of curcumin in niosome formulations. Data of EE were expressed as means±SD

Formulation	Surfactant		Curcumin (mg)	EE (%)
	Kind of span	Span : cholesterol ratio (mmol)		
A	20	7 : 3	20.00	28.43±0.002
B	60	7 : 3	20.00	61.22±0.004
C	80	7 : 3	20.00	36.00±0.002
D	20	7 : 2	20.00	26.11±0.002
E	60	7 : 2	20.00	58.90±0.003
F	80	7 : 2	20.00	30.05±0.001
G	20	8 : 3	20.00	36.15±0.003
H	60	8 : 3	20.00	50.41±0.002
I	80	8 : 3	20.00	14.58±0.002

Intercalation of cholesterol in the bilayers surface can decrease the entrapment volume during formulation, this phenomenon cause decrease entrapment efficiency[24]. Increasing interaction of solute surfactant with head group can increase numerous of entrapped drug, thus increases niosomes vehicle size. Using balance hydrophilic lipophilic of the drug affects the degree of entrapment[25, 26].

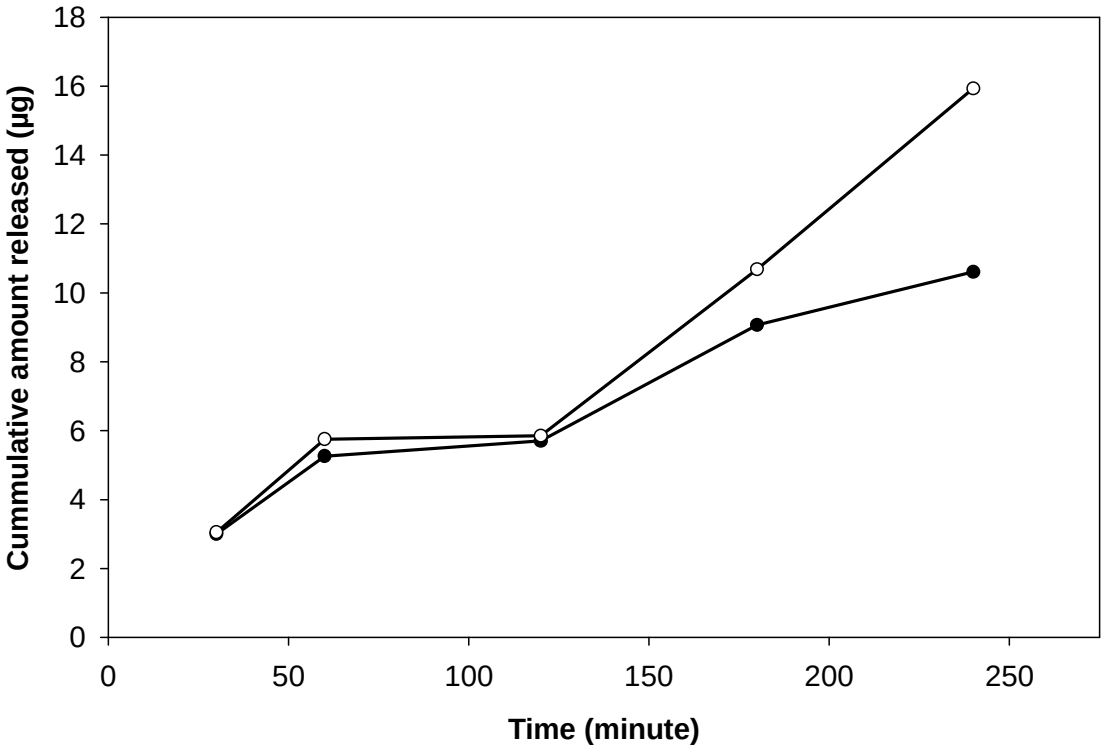

Figure 3. Penetration of curcumin in curcumin-niosome gel formulation on circumcision skin *ex vivo*. The data showed time courses of penetration of curcumin that formulated onto niosome gel compared curcumin unformulated through circumcision skin. The figure just showed formula B as the highest EE vs. negative control. Data are averages from three independent experiments. Notes: (---●---) control negative and (---○---) diclofenac gel

Formula B with the highest of EE show significant in ex vivo drug penetration after 180 min compared with unformulated curcumin.

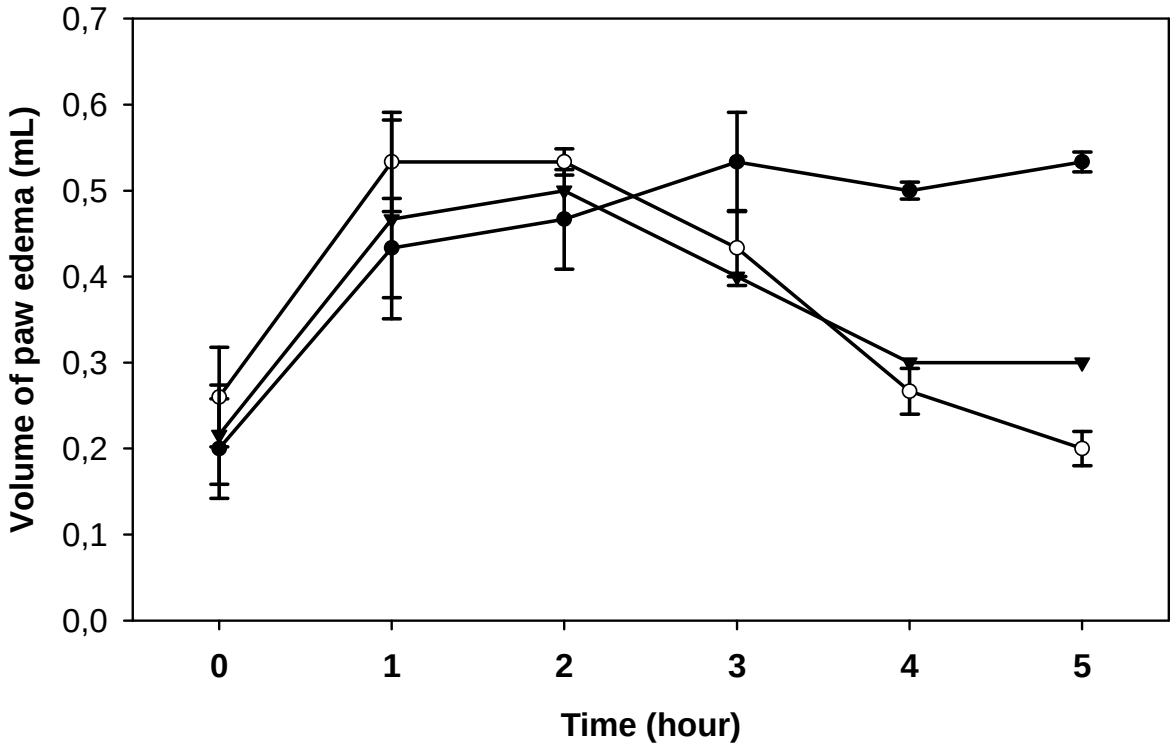

Figure 4. Anti-inflammation effect of curcumin-niosome gel on peptone-induced hind paw edema in rats. Each value represents the means±SD from five independent data (n= 3). Notes: (---●---) control negative, (---▼---) Formula B and (---○---) diclofenac gel®

Anti-inflammation effect

Inflammation is part of the complex biological response of body tissues injury stimuli, such as invasion of pathogens, necrotic cells, injury and damaged cells. The classical signs of acute inflammation are heat, redness, swelling, edema and loss of function[27]. Volume of edema on paw rat was used in this research as inflammation symptom.

Inflammation can be induced by unknown protein like peptone for bovine [28]. In inflammation protocol in pharmacological method usually induced by peptone or carrageenan. Cyclooxygenase sensitive to peptone thus suitable to investigate the effect of nonsteroidal anti-inflammation drugs [29].

Inflammation followed by metastatic of inflammatory cell and generate reactive oxygen species (ROS), reactive nitrogen species (RNS) and other free radicals, too. Superoxide anions, hydroxyl radical, singlet oxygen, hydrogen peroxide are most common of ROS that found in inflammation process [30, 31]. The largest effect antioxidant of curcumin can stabilize free radical species [32]. Curcumin-induced inhibition of cellular reactive oxygen species generation[33] and able to stabilize 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH*) has been reported [10].

CONCLUSION

In conclusion, the present study clearly demonstrated that curcumin-niosomal gel possess anti-inflammatory potency. The advantages that we found formula that has high-entrapped drug efficiency. For the further studies to establish the therapeutic value and elucidate the mechanism of action in the treatment of different inflammatory diseases.

REFERENCES

[1] Sharma S, Kulkarni SK, Chopra K. *Clinical and experimental pharmacology & physiology.* **2006**;33(10):p.940-945.
[2] Verghese J. *Flavour and Fragrance Journal.* **1993**;8(6):p.315-319.
[3] Esatbeyoglu T, Huebbe P, Ernst IM, Chin D, Wagner AE, Rimbach G. *Angewandte Chemie (International ed in English).* **2012**;51(22):p.5308-5332.
[4] Huang HC, Chang P, Lu SY, Zheng BW, Jiang ZF. *Journal of receptor and signal transduction research.* **20151**-8.
[5] Liu LB, Duan CN, Ma ZY, Xu G. *Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi = Chinese journal of integrated traditional and Western medicine / Zhongguo Zhong xi yi jie he xue hui, Zhongguo Zhong yi yan jiu yuan zhu ban.* **2015**;35(4):p.471-475.
[6] Guo H, Xu Y, Fu Q. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine.* **2015**;36(6):p.1-7.
[7] Guo Y, Li Y, Shan Q, He G, Lin J, Gong Y. *Int J Biochem Cell Biol.* **2015**;651-11.
[8] Al-Rubaei ZM, Mohammad TU, Ali LK. *Pakistan journal of biological sciences : PJBS.* **2014**;17(12):p.1237-1241.
[9] Naksuriya O, Okonogi S. *Drug discoveries & therapeutics.* **2015**;9(2):p.136-141.
[10] Ak T, Gulcin I. *Chem Biol Interact.* **2008**;174(1):p.27-37.
[11] Jurenka JS. *Alternative medicine review : a journal of clinical therapeutic.* **2009**;14(2):p.141-153.
[12] Stellar JE, John-Henderson N, Anderson CL, Gordon AM, McNeil GD, Keltner D. *Emotion (Washington, DC).* **2015**;15(2):p.129-133.
[13] Ploeger HE, Takken T, de Greef MH, Timmons BW. *Exercise immunology review.* **2009**;156-41.
[14] Chainani-Wu N. *Journal of alternative and complementary medicine (New York, NY).* **2003**;9(1):p.161-168.
[15] Menon VP, Sudheer AR. *Advances in experimental medicine and biology.* **2007**;595105-125.
[16] Handler N, Jaeger W, Puschacher H, Leisser K, Erker T. *Chemical & pharmaceutical bulletin.* **2007**;55(1):p.64-71.
[17] Skrzypczak-Jankun E, McCabe NP, Selman SH, Jankun J. *International journal of molecular medicine.* **2000**;6(5):p.521-526.
[18] Arunothayanun P, Bernard MS, Craig DQ, Uchegbu IF, Florence AT. *International journal of pharmaceutics.* **2000**;201(1):p.7-14.
[19] Radha GV, Rani TS, Sarvani B. *Journal of Basic and Clinical Pharmacy.* 2013;4(2):p.42-48.
[20] Marquet F, Payan J-P, Beydon D, Wathier L, Grandclaude M-C, Ferrari E. *Archives of Toxicology.* **2011**;85(9):p.1035-1043.
[21] Pyotr MK. *Chapter 3 Hydrophile-lipophile balance of surfactants*: Elsevier; **2000**.
[22] Kumar GP, Rajeshwarrao P. *Acta Pharmaceutica Sinica B.* **2011**;1(4):p.208-219.
[23] Shi B, Fang C, Pei Y. *Journal of pharmaceutical sciences.* **2006**;95(9):p.1873-1887.
[24] Szoka F, Jr., Papahadjopoulos D. *Annual review of biophysics and bioengineering.* **1980**;9467-508.

[25] Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, Kuotsu K. *Journal of Advanced Pharmaceutical Technology & Research.* **2010**;1(4):p.374-380.
[26] Kumar GP, Rajeshwarrao P. *Acta Pharmaceutica Sinica B.* **2011**;1(4):p.208-219.
[27] Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. *Clinical and Experimental Immunology.* **2007**;147(2):p.227-235.
[28] Bansal S, Bala M, Suthar SK, Choudhary S, Bhattacharya S, Bhardwaj V, Singla S, Joseph A. *European Journal of Medicinal Chemistry.* **2014**;80(0):p.167-174.
[29] Hotz-Behofsits CM, Walley MJM, Simpson R, Bjarnason IT. *Inflammopharmacology.* **2003**;11(4):p.363-370.
[30] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. *Antioxidants & redox signaling.* **2014**;20(7):p.1126-1167.
[31] Hakim J. *Comptes rendus des seances de la Societe de biologie et de ses filiales.* **1993**;187(3):p.286-295.
[32] Thayyullathil F, Chathoth S, Hago A, Patel M, Galadari S. *Free radical biology & medicine.* **2008**;45(10):p.1403-1412.
[33] Balasubramanyam M, Koteswari AA, Kumar RS, Monickaraj SF, Maheswari JU, Mohan V. *Journal of biosciences.* **2003**;28(6):p.715-721.