

The Effect of Niosomal System (Span 60-Cholesterol) on Diclofenac Sodium Preparation Characteristics and Diclofenac Sodium Preparation of Hydroxypropyl Cellulose Gel Base (HPC)

Adeltrudis Adelsa Danimayostu¹, Esti Hendradi², Tutiek Purwanti²

¹ Pharmacy Study Programme, Faculty of Medicine, University of Brawijaya

² Department of Pharmaceutics, Faculty of Pharmacy, Airlangga University

E-mail: mohabbat.elcha@gmail.com

ABSTRACT

Diclofenac sodium is a lipophilic drug. That characteristic makes it difficult to disperse well in hydrophilic gel base. The niosome with its vesicle system could overcome that low dispersibility. It affects penetration by reducing water loss in trans-epidermal layer and change lipid bilayer conformation. The present study was designed to investigate the effect of niosomal system (Span 60–Cholesterol) on preparation characteristics and diclofenac sodium penetration of hydroxypropyl cellulose (HPC) gel base. We examined three different formulas in HPC gel base. Formula III was made in niosomal system. Preparation characteristics were evaluated with organoleptic and acidity tests. Drug penetration was checked using *apparatus 5-paddle over disk* and 0.45 μm Milipore membrane impregnated with isopropyl myristate. The solution is phosphate buffer saline pH 7.4 ± 0.05 in temperature $37\pm 0.5^\circ\text{C}$. All of the data were evaluated based on one way ANOVA and continued with HSD test. It was concluded that niosomal system (span 60-cholesterol) has an influence in increasing pH value and penetration (based on flux value and permeability) of diclofenac sodium in HPC gel base.

Keywords: diclofenac sodium, niosomal system (span 60-cholesterol), hydroxypropyl cellulose, flux, permeability.

INTRODUCTION

Diclofenac sodium is one of *Non Steroidal Anti Inflammatory Drugs* (NSAIDs) which recently most used because of its analgesic and antiinflammatory activities for decades (Australian Government, 2014). Adverse effects of diclofenac per oral are upper GI bleeding, gastric ulcer or irritation happened in $\pm 20\%$ patients. As the result, 2% of them did not continue therapy (Hardman and Limbird, 2001). One of the recommendations to resolve the problem was by converting the dosage form using transdermal route. Transdermal delivery system becomes an attractive field to be an alternative for designing effective dosage form. The system could deliver either hydrophobic and lipophilic drugs. The advantage of transdermal systems are pain free, high compliance, avoid first pass metabolism and decrease side effects on GI tract (Paudel et al., 2010).

Gel is one of semisolid topical dosage form consists of dispersions of drugs molecules in an aqueous vehicle. It needs gelling agent to make its consistency become viscous like jelly. Hydroxypropyl cellulose is a cellulose derivatives synthetic macromolecules which has high molecular weight and construct a viscous and jelly system (Rowe et al., 2009; Allen et al., 2011).

In order to give pharmacology effect, drugs must be dissolved in base then released and penetrate through the skin

barrier to reach site of action (Barry, 1983). Diclofenac sodium is a lipophilic drug with partition coefficient of 13.4 (Florey, 1986). With such characteristics, diclofenac sodium could not well distributed in hydrophilic gel base. Diffusion, release, availability and penetration of the drug into skin were diminished, thus its pharmacological effect could not be achieved.

Some methods have been made to increase skin penetration, including vesicle system (Biju et al., 2006). Niosome is one of the vesicle systems. The composition consists of non ionic surfactant stabilized with cholesterol. Span-60 is a non ionic surfactant which frequently used as a component of niosome. The niosome can be portrayed as water inside non ionic surfactant bilayer, with or without cholesterol. Bilayer structure has been made from hydrophobic tail from monomer surfactant. The structure was act as a "protector" from the water in the middle and some hydrophilic heads. Cholesterol yields rougher bilayer and decrease niosome leakage risk (Choi & Maibach, 2005). Niosomes also gave better delivery for the drugs (Suresh and Kerunath, 2015).

The superaturation effect of niosomes in the surface of the skin induce the high accumulation of some lipophilic and hydrophilic drugs, such as ascorbic acid, tocopherol, minoxidil and ciprofloxacin (Mura et al., 2007; Varshosaz et al., 2014; Suresh and Kerunath, 2015). Those reasearchs could suggest that niosomes have a potential for optimal delivery of diclofenac sodium.

Niosome was able to make the drugs dispersed well in gel base and subsequent its diffusion and liberation had occurred easily. The hydrogels

capable to embe the water and niosome, thus enhance effectivity of diclofenac on local effects (Priprem et al., 2016). Niosome itself was also able to affect lipid bilayer skin structure conformation, diminished water losing on trans-epidermal layer and facilitated drugs penetration (Naresh et al., 1994; Shahiwala and Misra, 2002).

In this research, we would like to analyze the effect of niosomal system with the composition consist of diclofenac sodium–span 60–cholesterol (1:6:6) to improve penetration rate (flux and permeability) of diclofenac sodium. We also determined the formulation organoleptic characteristics (colour, odor, consistency) and pH.

MATERIALS AND METHODS

Diclofenac sodium (a gift from Dexa Medica, Corp); hydroxypropylcellulose (HPC, Nippon Soda Co, Ltd); propylenglycol (Brataco); Span 60 (Surya Dermato, Corp); cholesterol (Sigma); aquadest CO₂-free water (Dianum, Corp); and phosphate buffer solutions which composed of NaCl, KCl, Na₂HPO₄.12H₂O, KH₂PO₄ (E. Merck) were analytical grades.

Phosphate buffer saline (PBS) was prepared by mixing 8 grams NaCl; 0.194 grams KCl; 2.290 Na₂HPO₄.12H₂O; 0.196 grams KH₂PO₄. Those were taken and placed in 1,000 ml volumetric flask and aquadest was added to make the volume. Adjustments were made to reach pH target 7.4±0.05.

The calibration curve of diclofenac sodium was prepared in phosphate buffer pH 7.4. A stock solution 50 mg of diclofenac sodium in 500 ml of PBS was used to prepare 0.5; 1.0; 3.0; 5.0; 10.0; 15.0; 20.0; 25.0; dan 30.0 µg/mL solution.

Preparation of Niosomes.

Niosomes were prepared by using Reverse Phase Evaporation Technique (REV) based on the former research, Aliasgar (Shahiwala and Misra, 2002). The molar comparison of span 60: cholesterol was 1:1. The total comparison of diclofenac sodium: span 60: cholesterol was 1:3:3.

Characterization of Diclofenac Sodium Niosomes.

Vesicle dispersion was observed by Scanning Electron Microscopy for vesicle formation and morphology.

Determination of Diclofenac Sodium Entrapment Efficiency.

The 1 gram niosome suspension was diluted in PBS (1:4) then centrifuged at 6,000 ppm for 30 minutes. The clear part was pipetted as 1.0 ml and placed in 25.0 mL volumetric flask and PBS was added to make up the volume. The absorbance was measured to calculate diclofenac sodium amount dissolved or not entrapped by niosome (Cf) using UV-Vis spectrophotometer. The entrapment efficiency was calculated as $[(Ct-Cf)/Ct] \times 100\%$. Ct was the concentration of stock solution (Jufri et al., 2004; Patel, 2005).

Formulation.

We examined three different formulas in HPC gel base. Those were Formula I: diclofenac sodium and propylenglycol, Formula II: diclofenac sodium and propylenglycol mixed with span 60 and cholesterol. Formula III had the same component with formula II but it was made in niosomal system.

Monitoring of Excipient Effect and Homogeneity Test.

The purpose of the monitoring of excipient effect was diminished other absorbance which disrupted drugs absorbance evaluation. Homogeneity test

was used to ensure the drugs concentration on each sampling point (upper, middle and lower part of gel in the mortar) was similar.

The Evaluations and Analysis.

Evaluation of formulations were organoleptic (colour, odor and consistency), pH and penetration test. The data was analyzed using one way ANOVA method except organoleptic using descriptive analysis. The diffusion membrane used in this research was Milipore membran type HA (diameter 0,45 μm) impregnated with isopropyl myristate for one hour, then dried out and weighed until constant (Hendradi, 1995).

The devices for penetration rate test was based on USP 30/NF 25. It was apparatus 5 paddles over disk, consist of dissolution test assay with apparatus two paddles and completed with diffusion cell. The temperature on the dissolution tester was $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The paddle was turned around in 100 rpm and noted as minute-0. The samples (each 5 ml) were taken at minute 0.5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300.330, 360. Those samples were examined for absorbance using UV-Vis Spectrophotometer.

Linear regression was calculated using cumulative amount of drug versus sampling time (minutes). Based on Fick's diffusion law, the slope from the equation was the penetration rate (flux) of diclofenac sodium. Membrane permeability was calculated as flux divided by initial concentration of active compound on sample (Sinko and Singh, 2011)

RESULTS AND DISCUSSION

The niosome based on the former research had watery consistency. We tried to find another option and change the comparison into 1:6:6. Moreover, the

composition of cholesterol and span 60 (1:6:6) had never been tried.

The entrapment efficiency of diclofenac sodium–span 60–cholesterol (1:6:6) was 74.33%. It was higher than other ratio niosomal system (1:3:3) as 32.10%. From the evaluation of niosome morphology using Scanning Electron Microscopy (SEM), niosome specific shape was not seen. It could be due to inappropriate preparation of niosome evaluation.

There were no effect of excipients on diclofenac sodium absorbance. The excipients used in this study did not have chromophore cluster or conjugated double bond. The homogeneity test showed that the formulation was homogeneous. The requirement of homogeneity was the variation coefficient percentage of $\leq 6\%$.

Evaluation of organoleptic obtained: formula I was colourless, odorless, smooth and quite viscous; formula II was white milky,

odorless and very viscous; formula III was white milky, odorless, smooth and watery. The measurement of pH showed in Figure 1 is Formula I of 7.22 ± 0.07 , formula II of 7.06 ± 0.29 and formula III of 7.34 ± 0.06 . The pH value of formula III was outside pH skin range (4.5-6.8), so it tended to irritate skin.

We calculated flux value from the linear regression equation ($r=0.99983$) of some points started at steady state. As we can see in Figure 2, the condition was reached from minute of 30th and then flux value was calculated from regression equation slope cumulative amount of diclofenac sodium penetrated vs time start from minute of 30th until 360th. Flux mean values of every formulas were (Figure 3): formula I of 1.1367 ± 0.07 , formula II of 1.1447 ± 0.04 and formula III of 1.3728 ± 0.06 . Flux of formula II was higher than formula III. Those data indicated that niosome can increase diclofenac sodium flux from HPC gel base.

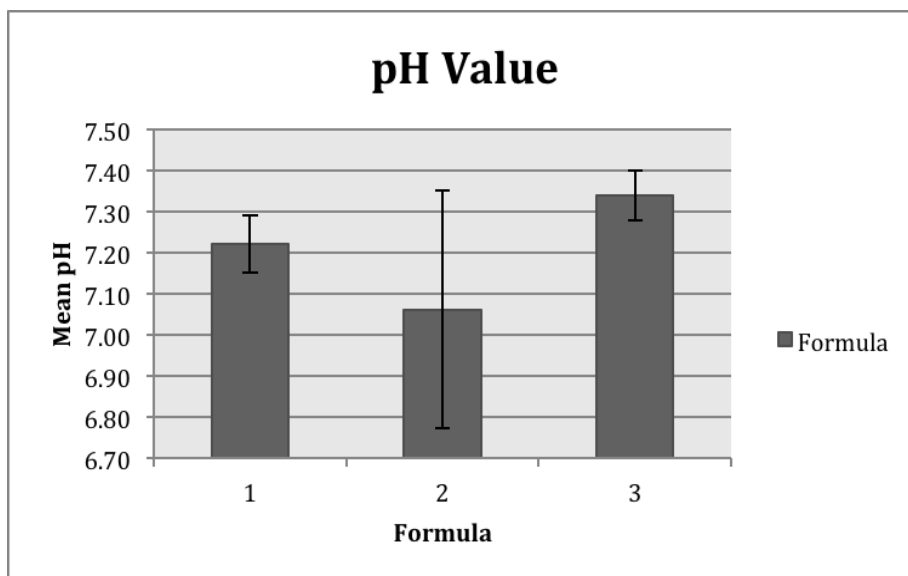


Figure 1. pH Value from Three Formulations

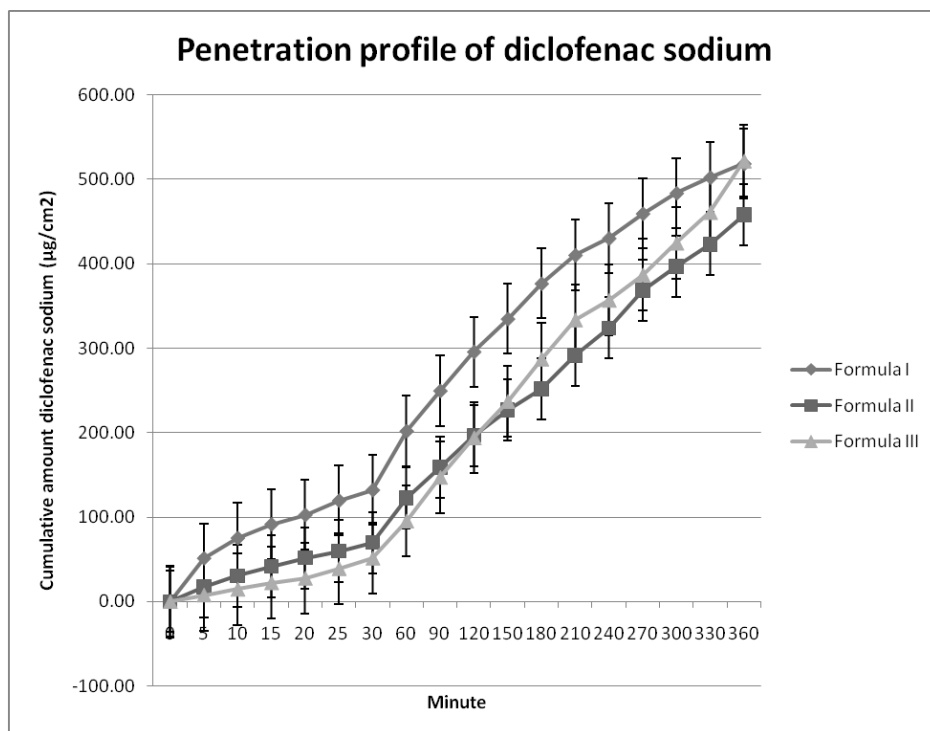


Figure 2. Penetration Profile of Diclofenac Sodium from Three Formulas

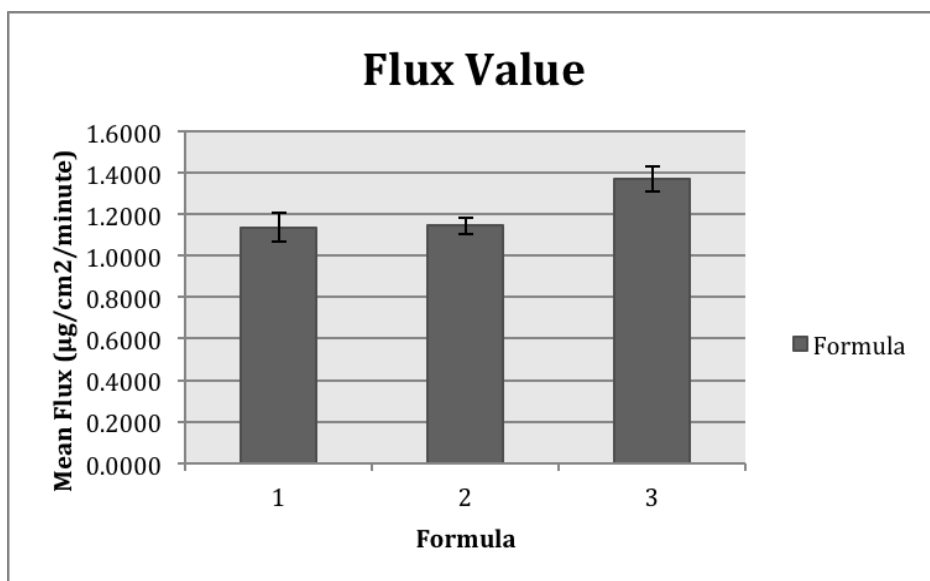


Figure 3. Flux of Diclofenac Sodium Penetrated

For the calculation of permeability, mean permeability of every formula is (Figure 4): formula I of $1.0320 \cdot 10^{-4} \pm 5.92 \cdot 10^{-6}$, formula II of $1.0289 \cdot 10^{-4} \pm 3.69 \cdot 10^{-6}$ and formula III of $1.3637 \cdot 10^{-4} \pm 6.15 \cdot 10^{-6}$. The permeability value of formula III was higher than others and differ significantly with formula II. It pointed out that niosomal system increased membran permeability.

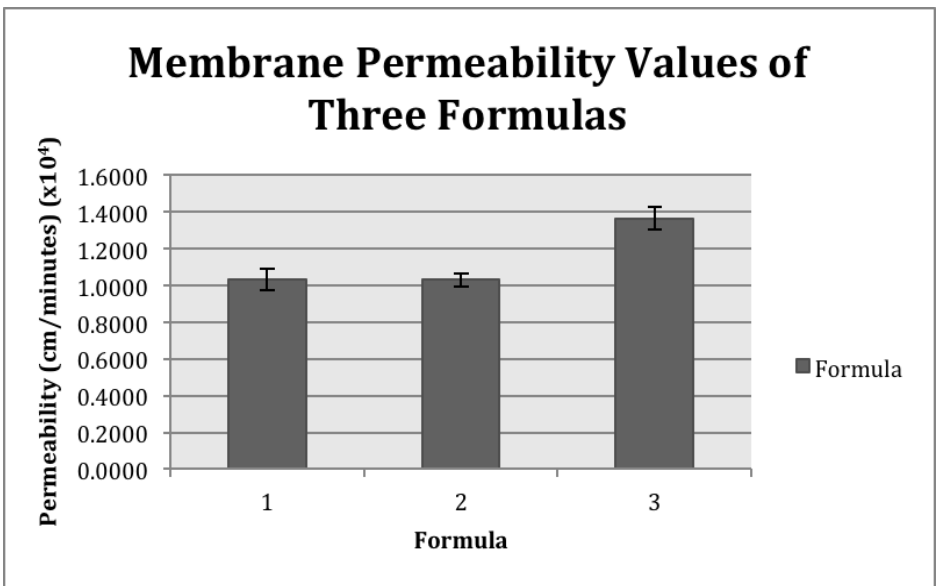


Figure 4. Membrane Permeability toward Diclofenac Sodium.

The data of pH, flux and permeability showed that the usage of niosomal system (diclofenac sodium-span 60-cholesterol = 1:6:6) on HPC gel base increase pH value, membrane permeability and penetration rate of diclofenac sodium in HPC gel base. Those elevations were due to good dispersion of drugs by niosomal system. It made the drugs release easily from gel base. Increasing of flux and permeability due to niosomal system prepared dispersion well so the drugs release easily from gel base. The impact was sufficient of drugs availability for optimal penetration. The next mechanism was niosome decreasing water losing at trans-epidermal layer and affecting conformation structure skin lipid bilayer.

For the first minutes, formula II gave higher line than formula III. It could be seen that penetration of formula II was faster. This could be due to the effect of propylenglycol in formula II. Propylenglycol, at first, it was used as a preservative. But, it obviously acted as a wetting agent. Propylenglycol also dissolved diclofenac sodium so the drug dispersion was better.

The difference of mixing with propylenglycol was the cause of higher cumulative amount of diclofenac sodium in formula II at the first minutes. In formula II, propylenglycol wet diclofenac sodium. Meanwhile, propylenglycol had been mixed all in the gel base in formula III. The direct contact between diclofenac sodium and propylenglycol made a better dispersion. Unfortunately, this effect could not make high cumulative amount of diclofenac sodium. As we could see in Figure 2, the cumulative amount of formula II was less than formula III.

In formula II, span 60 and cholesterol were only mixed with the other component. On that way, they made the gel was at hydrophobic ambiance. Diclofenac sodium's affinity with span 60 and cholesterol would be stronger so the drug was less capable to release optimally. It had an effect in availability and penetration. Even though span 60 had hydration effect which could help penetration effect, but its ability was not supporting significantly. Moreover, the drug availability was less. Overall, formula III had increasing

penetration rate of 19.93% and permeability 32.54% compared with formula II.

CONCLUSION

It can be concluded that the niosomal system (diclofenac sodium-span 60-cholesterol=1:6:6) with its entrapment efficiency of 74.33%; it had influence in increasing pH value and penetration (based on flux value and permeability) of diclofenac sodium in HPC gel base. That formulation also gave white milky, odorless, smooth and watery consistency characteristics. The pH value of formula III was high, 7.34. We suggest further research to give the formulation with normal pH value for human skin. In addition, the morphology evaluation of niosomes was very important. Optimizing SEM method preparation was needed for niosomes evaluation.

ACKNOWLEDGEMENT

Authors are grateful to Dexa Medica, Corp, Palembang, Indonesia for providing gift sample of diclofenac sodium.

REFERENCES

- Australian Government Department of Health. 2014. *Safety Review of Diclofenac*. Commonwealth of Australia.
- Allen, L. V. Popovich, N. G., and Ansel, HC. (Eds). 2011. *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. 9th edition*. Baltimore: Lippincott Williams & Wilkins.
- Barry, B. W. 1983. *Dermatological Formulation Percutaneous Absorption*. New York: Marcel Dekker Inc.
- Biju, S. S., Sushama,T., Mishra, P. R., & Khar, R. K. 2006. Vesicular Systems: An Overview. *Indian J Pharma Sci.* 68 (2): 141-153.
- Choi, M. J., and Maibach, H. I. 2005. Liposomes and Niosomes as Topical Drug Delivery Systems. *Skin Pharmacology and Physiology.* 18 (5): 209-219.
- Florey, K. 1986. *Analytical Profiles of Drug Substance*. Orlando: Academic Press Inc.
- Hardman, J. G., and Limbird, L. E. 2001. *The Pharmacological Basis of Therapeutics. 10th edition*. United States: The McGraw Hill. p. 709 -710.
- Hendradi, E. 1995. *Kinetika dan Mekanisme Transport Beberapa Antihistamin Melewati Membran Lipid*. Thesis. Yogyakarta.
- Jufri, M., Anwar, E., and Djajadisastra, J. 2004. Pembuatan Niosom Berbasis Maltodekstrin DE 5-10 dari Pati Singkong (*Manihot utilissima*). *Majalah Ilmu Kefarmasian, I* (1): 10-20.
- Mura, S., Pirot, F., Manconi, M., Falson, F., and Fadda, A. M. 2007. Liposomes and niosomes as potential carriers for dermal delivery of minoxidil. *Journal of Drug Targeting, February* 15(2): 101-108.
- Nares, R. A. R., Pillai, G. K., Udupa, K., and Chandrashekar, G. 1994. Antiinflammatory Activity of Niosome Encapsulated Diclofenac Sodium in Arthritic Rats. *Indian J Pharmacol.* 26: 46-48.
- Patel, R. 2008. Niosome: An Unique Drug Delivery System. Retrieved From the website: <http://www.pharmainfo.net/reviews/niosome-unique-drug-delivery-system>.
- Paudel, K. S., Milewski, M., Swadley, C. L., Brogden, N. K., Ghosh, P., and Stinchcomb, A. L. 2010. Challenges and opportunities in

Danimayostu et al. : The Effect of Niosomal System (Span 60-Cholesterol) on

- dermal/transdermal delivery. *Their Deliv. July*, 1(1): 109-131.
- Priprem, A., Janpim, K., Nualkaew, S., and Mahakunakorn, P. (2016). Topical Niosome Gel of *Zingiber cassumunar* Roxb. Extract for Anti-inflammatory Activity Enhanced Skin Permeation and Stability of Compound D. *AAPS PharmSciTech* 17 (3): 631–639.
- Rowe, R. C., Sheskey, P. J., and Quinn, M. E. (Eds). 2009. *Handbook of Pharmaceutical Excipients. 6th edition*. London: Pharmaceutical Press.
- Shahiwala, A., and Misra, A. 2002. Studies in Topical Application of Niosomally Entrapped Nimesulide. *J Pharm Pharmaceut Sci.*, 5 (3):220 –225.
- Sinko, P. J., and Singh, Y. (2011). *Martin's Physical Pharmacy and Pharmaceutical Sciences. 6th edition*. Baltimore: Lippincott Williams & Wilkins.
- Suresh, R. V., and Kerunath, K. P. 2015. Formulation and Evaluation of Novel Anti-Bacterial Ciprofloxacin Loaded Niosomal Cream. *Int. Res. J. Pharm.* 6 (8). 519-527.
- Varshosaz, J., Taymouri, S., Pardakhty, A., Asadi-Shekaari, M., and Babaei, A. 2014. Niosomes of Ascorbic Acid and Tocopherol in the Cerebral Ischemia-Reperfusion Model in Male Rats. *BioMed Research International*. 2014: 1-9.