The Pharma Innovation Journal 2023; 12(12): 3780-3784

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 3780-3784

www.thepharmajournal.com
Received: 08-09-2023
Accepted: 17-11-2023

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Evaluation of the *in vivo* anti-inflammatory activity of piroxicam-loaded ethosomal gel for transdermal delivery

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Abstract
Ethosomes are pliable phospholipids nanovesicles, with high ethanol content (20-45%) and water for dermal and transdermal delivery. The present study aimed to evaluate the anti-inflammatory properties of piroxicam-loaded ethosomal gel. The ethosomal formulations were prepared using the hot method. Six batches of piroxicam ethosomal gels (EG1-EG6) were prepared by incorporating the ethosomal suspensions into carbopol gel bases and two extra batches of plain gels without ethosomal suspensions (PG1and PG2) were made. All 8 batches were evaluated for physicochemical properties, and *in vivo* anti-inflammatory activity. The *in vivo* anti-inflammatory potentials of the piroxicam ethosomal gels were compared with those of the plain gels. The physicochemical evaluation showed gel spreadability and extrudability values of 2.05-5.00 g.cm/sec and 9.60-38.46% respectively. Plain gels gave higher viscosity values than ethosomal gels. Formulation EG2 exhibited significantly higher (p<0.05) anti-inflammatory activity in comparison with the control. Also the formulations containing the ethosomal vesicle (EG1 and EG2) showed higher anti-inflammatory activity than the reference plain gel PG2. The optimized formulation EG2 (0.5% Phospholipid and 40% ethanol), exhibited better profile with a percentage edema inhibition of 50.9% as against 40.48% for the reference formulation PG2.Ethosomes could be an efficient carrier for the transdermal delivery of piroxicam in the treatment of inflammation.

Keywords: Ethosomes, transdermal delivery, percentage inhibition, anti-inflammatory activities, phospholipid

Introduction
Inflammation serves as a natural defense mechanism triggered by an assault on the body, aimed at isolating and repairing tissue damage. Its protective role is evident in the innate defense of the body and clinically manifests through four primary indicators: redness, heat, pain, and swelling (Khedir *et al*., 2016) [1].

Piroxicam, a nonsteroidal anti-inflammatory drug (NSAID) with the chemical name 4-hydroxy-2-methyl-2-H-1,2-benzothiazine-1-(N-(2-pyridinyl) carboxamide)-1,1-dioxide, is employed in treating various inflammatory conditions, including rheumatoid arthritis and osteoarthritis. It functions as a non-selective cyclooxygenase (COX) inhibitor, possessing anti-inflammatory, analgesic, and antipyretic properties. However, due to the occurrence of side effects in around 30% of patients receiving a daily dose of 20 mg, such as upper abdominal pain and gastrointestinal mucosa ulceration, its oral use is restricted. Transdermal delivery of NSAIDs offers an effective strategy to avoid adverse effects on the gastrointestinal tract while enhancing patient compliance (Goosen *et al*., 1998; Boudreau and Beland, 2006; Chaudhary *et al*., 2013) [2-4].

It is common knowledge that when drug molecules traverse the skin, they undergo two distinct processes. This begins with drug penetration through the stratum corneum, followed by drug diffusion into deeper tissues. The rate and extent of drug transport through the stratum corneum depend on factors such as size, log P, ionic strength, hydrogen bonding ability, and the physicochemical properties of the vehicle (Roberts and Cross, 1999) [5].

Numerous passive and active methods have been proposed to increase skin permeability for transdermal drug delivery. These methods include penetration enhancers, vesicles, iontophoresis, supersaturated systems, electroporation, phonophoresis, the use of microneedles, and jet injectors. One of the most practical approaches for facilitating drug transport through the skin involves utilizing vesicle formulations as a delivery system for the

skin. One particularly effective method for transdermal drug delivery involves the use of vesicle formulations (Santoyo and Ygartua, 2000; Shin *et al.*, 2000; Curdy *et al.*, 2001; Cheong and Choi, 2002; Pénzes *et al.*, 2005) [6-10].

Ethosomes are pliable lipid vesicles primarily composed of phospholipids, with a relatively high ethanol content (20-45%) and water (Saquib *et al.*, 2018) [11]. Ethanol serves as an enhancer that imparts flexibility, enabling rapid permeation through the deeper layers, such as the stratum corneum of human skin (Kapoor *et al.*, 2011; Li *et al.*, 2012) [12-13]. Ethanol enhances the flexibility and fluidity of lipids, loosening the tight junctions of the stratum corneum, thereby increasing skin permeability and facilitating the penetration of disorganized lipid bilayers. It also aids in the release of drugs into the systemic circulation (Mahmood *et al.*, 2018) [14].

The transdermal device functions as a membrane-mediated system, offering an alternative route for the delivery of one or more active ingredients. This delivery system has gained recognition as a promising method to bypass the hepatic metabolism of drugs, ensuring patient compliance and providing sustained drug activity. Compared to conventional drug delivery methods, the transdermal drug delivery system offers several advantages, including lower drug dosages, enhanced patient compliance, convenience, avoidance of gastrointestinal disturbances, and first-pass metabolism (Kurmi *et al.*, 2017) [15]. Applying anti-inflammatory agents transdermally at the site of inflammation can mitigate their systemic side effects, thereby enhancing patient adherence and therapeutic outcomes. The aim of this research therefore was to assess the *in vivo* anti-inflammatory efficacy of piroxicam-loaded ethosomal gel for transdermal administration by leveraging a combination of nanotechnology and transdermal drug delivery to enhance the delivery of piroxicam, maximizing its anti-inflammatory potential.

Materials and Methods

Materials: Piroxicam powder (Central Drug House, New Delhi, India), Propylene glycol (JHD, China), Soy lecithin (Guangdong Guanghua Sci-Tech. Co. Ltd, China), Methyl and propylparaben (Qingdao Che., China), Carbopol 940 (JHD, China), Triethanolamine (JHD, China) and Ethanol (JDH, China).

Ethical approval

Approval for the use of animals in this study was granted by the Research and Ethics Committee of the Faculty of Basic Medical Sciences of the Delta State University, Abraka, Nigeria with approval no: REC/FBMS/DELSU/22/156

Preparation of ethosomal vesicles: Ethosomal vesicles (E1-E6) were prepared with slight modifications of the hot plate method (16), according to the quantities stated in Table 1 below. The drug concentration was fixed at 0.08% w/w. the soy lecithin (phospholipid) and ethanol concentrations were in the range of 0.5-1% w/w and 20-60% w/w respectively. An accurately weighed quantity of the piroxicam was dissolved in propylene glycol. The soy lecithin was dissolved in a mixture of ethanol and propylene glycol at 40 ºC in another beaker. The drug solution was then added slowly to soy lecithin dispersion of water at 40 ºC in a vessel and stirred for 30 minutes at 1,700 rpm using a magnetic stirrer. Thereafter 5 ml of water was added in small quantities at a time to the formulation using a 22G syringe needle. The evenly dispersed ethosomal vesicle formed for each batch was stored in the refrigerator at 4 ºC for further study.

Table 1: Composition of suspension of ethosomal vesicle

Batch	Piroxicam (%w/v)	Lecithin (% w/v)	Ethanol (% v/v)	Propylene glycol (%v/v)	Water (%v/v)
EV1	0.08	0.5	20	20	100
EV2	0.08	0.5	40	20	100
EV3	0.08	0.5	60	20	100
EV4	0.08	1	20	20	100
EV5	0.08	1	40	20	100
EV6	0.08	1	60	20	100

Preparation of ethosomal gel

The gels were prepared by dispersion method using carbopol 940 as the gelling agent according to the quantities stated in Table 2. Gels were prepared by dispersing carbopol in distilled water to hydrate and swell for 24 h. The polymer solution was then neutralized by the addition of triethanolamine (in a stoichiometric ratio of carbopol to triethanolamine of 1:1.5) dropwise with frequent stirring. Ethosomal vesicular suspensions EV1-EV6 were added and mixed properly to form gels EG1-EG6. Mixing was done until a transparent gel was formed. Paraben was added as a preservative. Two extra batches PG1 and PG2 were prepared without incorporating piroxicam ethosomes in them, making them just plain gels. The prepared gels were filled in glass vials and stored at 4-8 ºC for further evaluation.

Table 2: Composition of piroxicam ethosomal gel

Batch	Carbopol (% w/v)	TEA (ml)	Piroxicam (%w/v)	Ethosomal vesicle (ml)	Paraben (% v/v)	Water to (% v/v)
EG1	1	1.3	0.04	50	0.1	100
EG2	1	1.3	0.04	50	0.1	100
EG3	1	1.3	0.04	50	0.1	100
EG4	2	2.6	0.04	50	0.1	100
EG5	2	2.6	0.04	50	0.1	100
EG6	2	2.6	0.04	50	0.1	100
PG1	1	1.3	0.04	-	0.1	100
PG2	2	2.6	0.04	-	0.1	100

TEA: Triethanolamine

Evaluation of ethosomal gel

pH measurement

A 1 g sample of ethosomal gel was diluted to 100 ml with de-ionized water in a volumetric flask. The pH of the resulting 1% dispersion was measured using a pH meter. Determinations were carried out in triplicates and the mean result was recorded.

Spreadability study

The spreadability of the gel was determined by measuring the spreading diameter of 0.2 g of gel between two horizontal plates (20 cm x 20 cm) after one minute. The standard weight applied on the upper plate was 300 g.

Viscosity measurement

The rheological behaviour of the formulations was evaluated by viscosity measurement. The viscosity of the formulated gels was determined using a Brookfield viscometer CAP-2000. Test samples were weighed in a clean and dry 250 ml beaker, and the viscosity of the test samples was determined using spindle 4 following standard operating procedure at 12 rpm. Samples were measured at 27 ± 1 °C (Akanksha *et al.*, 2009) [17].

Extrudability study

Extrudability was determined by measuring the amount of gel extruded out of a collapsible tube on the application of a 500 g load for 1 min. The percentage extrudability was calculated as the ratio of the amount of gel extruded to the amount of gel in the tube (Okafo *et al.*, 2022) [18].

***In vivo* anti-inflammatory activity**

Male Wistar rats weighing approximately 120 g kept at the laboratory animal house of the Department of Pharmacology, Delta State University, Abraka, were used. The animals were maintained under standard environmental conditions and had free access to a standard diet and water. Anti-inflammatory activity was measured using the carrageenan-induced rat paw oedema assay. Piroxicam ethosomal gels (formulation EG1 and EG2) and piroxicam plain gel (formulation PG2) were administered to three groups of 6 rats each. The fourth group which is the control group were simply given 1% carrageenan suspension in normal saline solution and was injected into the sub-plantar tissue of the right hind paw.

One hour after the administration of EG1, EG2 and PG2, 0.1 ml of 1% carrageenan suspension in saline solution were injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly intervals for 4 hours. Anti-inflammatory activity was measured as the percentage reduction in oedema level when the drug was present relative to control (Duffy *et al.*, 2001; Adeyemi *et al.*, 2002) [19-20].

Statistical analysis

The data was presented as mean ± standard deviation. Statistical difference between the mean values were determined by one-way analysis of variance (ANOVA). A p-value < 0.05 was considered significant.

Results

Physicochemical characteristics of piroxicam ethosomal gel

The physicochemical properties of piroxicam ethosomal gel are presented in Table 3 below. The pH of the formulated gels were between 5.65 and 6.61 which fell within the acceptable pH range for topical preparations of 4 - 6. Topical products should be acidified and possess a pH in the range of 4 to 6 because an increase in pH increases the dehydrative effect, irritability and propionic bacteria count (Luki´c *et al.*, 2001; Baranda *et al.*, 2002) [21, 22].

The result also reveal that all the gels were clear and homogenous. The Ethosomal gels exhibited good spreadability and extrudability than the plain gels with batches EG1-EG3 showing the higher Spreadability and extrudability values of 4.1-5.0 g.cm/sec and 20.36-38.46% respectively. However the plain gels PG1 and PG2 gave much higher viscosity values of 41,500 and 69,500 respectively as against Ethosomal gels with values ranging from 11,200-17,800. The addition of the suspension of ethosomal vesicles containing phospholipid and ethanol may be responsible for the reduction in viscosity of the Ethosomal gels.

Table 3: Physicochemical properties of the ethosomal gel

Batch	Clarity	Homogeneity	Spreadability (cm)	Extrudability (%)	pH	Viscosity (mPa s)
EG1	Clear	Very good	4.40 ± 0.21	35.71	6.42	13,600
EG2	Clear	Very good	5.00 ± 0.20	38.46	6.61	11,300
EG3	Clear	Very good	4.10 ± 0.24	20.36	6.22	11,200
EG4	Clear	Very good	3.45 ± 0.22	13.06	6.18	17,800
EG5	Clear	Very good	3.75 ± 0.22	17.18	5.81	15,500
EG6	Clear	Very good	3.25 ± 0.24	9.60	5.65	17,000
PG1	Clear	Very good	2.05 ± 0.24	15.36	5.81	69,500
PG2	Clear	Very good	3.80 ± 0.23	33.25	5.86	41,500

Anti-inflammatory activity of the ethosomal gel in rats

The result of the anti-inflammatory activity expressed as the percentage of edema inhibition relative to the control is presented in Figure 1. The percentage of edema inhibition after 4 h was between 42.86 and 50.9% with formulation E2 showing the highest percentage of edema inhibition of 50.9% as against 40.9% activity of the plain gel PG2 having the same concentration of piroxicam (0.04%). This is similar to the study by Umesh *et al.*, 2021 where piroxicam gel was evaluated and the percentage inhibition was about 58% (Umesh *et al.*, 2021) [23].

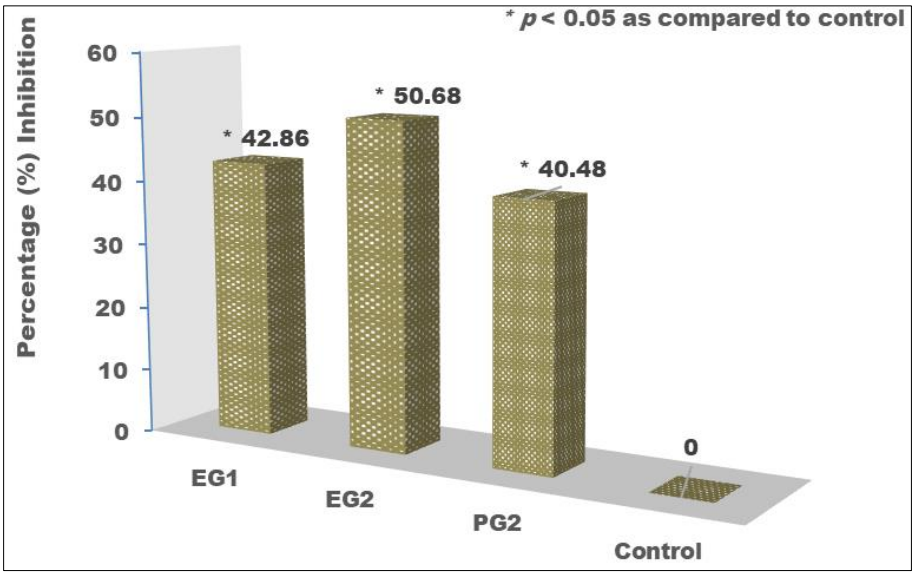

Fig 1: Percentage inhibition of carrageenan induced paw edema in rats after treatment with piroxicam ethosomal and plain gels. EG1 (0.04% piroxicam, 0.25% Lecithin, 20% ethanol); EG2 (0.04% piroxicam, 0.25% Lecithin, 40% ethanol); PG2 (0.04% piroxicam)

Discussion

It was also observed that the pH increased with decrease lecithin concentration, ethosomal gels EG1-EG3 with lecithin concentration of 0.5% showed higher pH values of 6.22-6.61, while EG4-EG6 with lecithin concentration of 1% gave lower pH values of 5.65-6.18. This relationship between pH and lecithin concentration is similar to the study on piroxicam nano ethosomal formulation by another researcher (Kazemi *et al.*, 2019) [24]. The plain gels had much higher viscosities than the ethosomal gels despite having corresponding concentrations of the gelling agent, carbopol. The incorporation of ethosomal vesicular suspension is most likely responsible for a reduction in the viscosity of the ethosomal formulations.

Carrageenan-induced hind paw Oedema is the standard experimental model for acute inflammation with high degree of reproducibility. Carrageenan is the agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effects (Winter *et al.*, 1963) [25]. Carrageenan-induced Oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h (Vinegar *et al.*, 1969) [26].

It can be seen from Figure 1 that formulations EG1, EG2 and PG2 exhibited significantly higher (p<0.05) anti-inflammatory activity in comparison with the control. Also the formulations containing the ethosomal vesicle (EG1 and EG2 showed higher activity than the reference plain gel PG2 without ethosomes). This may be due to increase *in vivo* absorption and penetration in the presence of the ethosomal vesicle (ethanolic vesicle) as corroborated by studies of other researcher that showed the efficient capability of ethanolic vesicles in localizing the drug in the site of inflammation in contrast to conventional dosage forms. A study proved valsartan-loaded ethosomes significantly superior in terms of drug release and increment in bioavailability (Ahad *et al.*, 2013) [27].

Ethosomal gel EG2 (containing 0.5% of Phospholipid (Lecithin) and 40% ethanol) exhibited higher anti-inflammatory activity than plain gel. This result is similar to earlier findings on *Mangifera indica*-loaded ethosomal gel where ethosomal gel formulations gave better anti-inflammatory activity compared to the blank gel (Sireesha *et al.*, 2018) [28].

Conclusion

In conclusion piroxicam-loaded ethosomes were successfully formulated and evaluated. Piroxicam-loaded ethosomal gels showed overall superior activity compared to the plain gel formulation for skin permeation, and anti-inflammatory activity. Ethosomes could possibly be employed as a drug carrier for the transdermal delivery of piroxicam for the treatment of inflammation

Acknowledgements

The authors appreciate the effort made by the technologists Mr. Joshua Agboun and Mr. Lawrence Ewhre of the Department of Pharmaceutics and Industrial Pharmacy and the Department of Pharmacology and Toxicology respectively.

References

1. Khedir SB, Mzid M, Bardaa S, Moalla D, Sahnoun Z, Rebai T. *In vivo* Evaluation of the Anti-Inflammatory Effect of *Pistacia lentiscus* Fruit Oil and Its Effects on Oxidative Stress. Evid. Based Complementary Altern. Med; c2016.
2. Chaudhary H, Rohilla A, Rathee P, Kumar V. Optimization and formulation design of carbopol loaded Piroxicam gel using novel penetration enhancers. Int. J Bio Macromol. 2013;55:246-53.
3. Goosen C, du Plessis J, Muller DG, Janse van Rensburg LF. Correlation between physicochemical characteristics, pharmacokinetic properties and transdermal absorption of NSAID's. Int. J Pharm. 1998;163:203-9.
4. Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of Aloe Barbadensis (Miller), Aloe vera. J Environ. Sci. Health. C. Environ. Carcinog. Ecotoxicol. Rev. 2006;24:103-54.
5. Roberts MS, Cross SE. Percutaneous absorption of topically applied NSAIDs and other compounds: Role of

solute properties, skin physiology and delivery systems. Inflammopharmacology. 1999;7:339-350.
6. Cheong HA, Choi HK. Enhanced percutaneous absorption of piroxicam via salt formation with ethanolamines. Pharm Res. 2002;19:1375-1380.
7. Santoyo S, Ygartua P. Effect of skin pretreatment with fatty acids on percutaneous absorption and skin retention of piroxicam after its topical application. Eur. J Pharm Biopharm. 2000;50:245-250.
8. Pénzes T, Blazsó G, Aigner Z, Falkay G, Eros I. Topical absorption of piroxicam from Organogels – *in vitro* and *in vivo* correlations. Int. J Pharm. 2005;298:47-54.
9. Shin SC, Cho CW, Oh IJ. Enhanced efficacy by percutaneous absorption of piroxicam from the poloxamer gel in rats. Int. J Pharm. 2000;193:213-218.
10. Curdy C, Kalia YN, Naik A, Guy RH. Piroxicam delivery into human stratum corneum *in vivo*: Iontophoresis versus passive diffusion. J Control Release. 2001;76:73-79.
11. Saquib RZ, Neeraj U, Surendra D, Sudhir KR, Prabhat J, Geeta P. Ethosome: A novel vesicular carrier for transdermal drug delivery. J Drug Del. Ther, 2018;8(6):318-326.
12. Kapoor D, Patel M, Singhal M. Innovations in Transdermal drug delivery system. Int. J Pharm. Sci. 2011;1(1):54-61.
13. Li G, Fan Y, Fan C, Li X, Wang X, Li M, *et al*. Tacrolimus-loaded ethosomes: Physicochemical characterization and *in vivo* evaluation. Eur. J Pharm Biopharm. 2012;82(1):49–57.
14. Mahmood S, Mandal UK, Chatterjee B. Transdermal delivery of raloxifene HCl via ethosomal system: Formulation, advanced characterizations and pharmacokinetic evaluation. Int. J Pharm. 2018;542(1-2):36–46.
15. Kurmi B Das, Tekchandani P, Paliwal R, Paliwal SR. Transdermal Drug Delivery: Opportunities and Challenges for Controlled Delivery of Therapeutic Agents Using Nanocarriers. Curr Drug Metab. 2017;18(5):481-95.
16. Ashoniya S, Meenakshi C. Ethosome as a vesicular carrier for enhanced transdermal delivery of ketoconazole-formulation and evaluation. J Pharm Cosmetol. 2011;1(3):485-489.
17. Akanksha D, Vikas G, Neetesh KJ, Shalendra S, Neelam B, Dinesh KJ. Formulation and Evaluation of Neomycin Sulphate Ointment containing Natural Wound Healing Agent Curcuma longa, Int. J Pharm. Sci. Drug Res. 2009;1:105–118.
18. Okafo SE, Alalor CA, Israel NC, Agbamu E. Evaluation of Naproxen-Loaded Emulgels Formulated Using Moringa Oleifera Seed Oil. Int. J. Biol. Pharm. Allied Sci. 2022;11(11):5315-5331.
19. Adeyemi OO, Okpo SO, Ogunti OO. Analgesic and anti-inflammatory effect of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia. 2002;73:375-380.
20. Duffy JC, Dearden JC, Rostron C. Design, Synthesis and biological testing of a novel series of anti-inflammatory drugs. J Pharm. Pharmacol. 2001;53:1505-1514.
21. Luki´c M, Panteli´c I, Savi´c SD. Towards Optimal pH of the Skin and Topical Formulations: From the Current State of the Art to Tailored Products. Cosmetics. 2021;8:69.
22. Baranda L, Gonzalez-Amaro R, Torress-Alvarez B, Alvarez C, Ramirez V. Correlation between pH and irritant effect of cleansers marketed for dry skin, Int. J Dermatol. 2002;41:494-499.
23. Umesh JT, Sayali RP, Gunesh DN, Shital SD, Sneha LS. Formulation and critical evaluation of piroxicam gel. J Pharm. Innov. 2021;10(3):89-94.
24. Kazemi M, Mombeiny R, Tavakol S, Keyhanvar P, Mousavizadeh K. A combination therapy of nanoethosomal piroxicam formulation along with iontophoresis as an anti-inflammatory transdermal delivery system for wound healing. Int. Wound J. 2019;16:1144–1152.
25. Winter CA, Risley EA, Nuss GW. Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 1963;111:544-7.
26. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. J Pharmacol. Exp. Ther. 1969;166:96-103.
27. Ahad A, Aqil M, Kohli K, *et al*. Enhanced transdermal delivery of an anti-hypertensive agent via nano ethosomes: statistical optimization, characterization and pharmacokinetic assessment. Int. J Pharm. 2013;443:26-38.
28. Sireesha K, Sailaja RP, Bala TS, Prakash DV. Preparation and Evaluation of Mangifera Indica Loaded Ethosomal Gel for Anti-Inflammatory Activity in Animal Model. Int. J Ayurvedic Herb. Med. 2018;8(1):3089-3098.