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SYNERGISTIC AND SUSTAINED ANTI-INFLAMMATORY ACTIVITY OF GUGUUL WITH THE IBUPROFEN: A PRELIMINARY STUDY

*SHEKHAR VERMA, ANUREKHA JAIN *, AND V.B.GUPTA*

B.R.Nahata College of Pharmacy, Mandsaur (M.P.), 458001 India

**** Corresponding author*** anurekha_jain@yahoo.com

KEY WORDS

Guggul, Guggulipid, Drug Carrier ,anti-inflammatory and ibuprofen

ABSTRACT

Guggulosomes prepared using guggul by bath sonication and trituration methods were studied for optical microscopy, *in vitro* release profile, entrapment efficiency of drugs and anti-inflammatory activity. Average particle size determined was between 0.1 µm to 0.2 µm. Guggulosomes sustained the drug release for a period of 9 hrs and more in bath sonification method. Ibuprofen loaded guggulosomes showed maximum entrapment efficiency of 26.0 % in bath sonication method. Anti-inflammatory study of Guggulosomes revealed that it is having more efficacy than Ibuprofen and guggul shows that guggulosomes produced synergistic effect with ibuprofen. Thus from the studies conducted so far we can conclude that guggul could serve as a carrier for entrapping drugs and shows sustained release action.

INTRODUCTION

Over the past few decades, there has been growing interest in developing new drug delivery system that offers numerous advantages compared to conventional dosage forms. Such systems often use macromolecules as carriers for the drugs[1]. Carriers are one of the most important entities essentially required for successful transportation of the loaded drugs. They act as drug vectors, which sequester, transport and retain drug *en rout* and elute or deliver it within or in the vicinity of target. An ideal drug carrier engineered, as a controlled device should have many properties, such as, carrier should be non-toxic, non-immunogenic and biodegradable particulate, it must recognize specifically and selectively the target cell. The importance of lipids as drug carrier is well established to interact with biological targets or to release the drug in the proximity of target cell lines demanding optimal pharmacological action[2].

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Guggul is an oleo-gum-resin obtained from plant *Commiphora wightii* or *Commiphora mukul* (Family-Burceacea)[3]. Traditionally guggul has been used in Ayurvedic medicines in the form of Yograjguggulu vati, Kaisorguggulu vati, Singhnadguggulu vati and

Triphalaguggulu for treatment of many ailments including rheumatism, arthritis, hyperlipidemia, obesity, inflammation, atherosclerosis, wrinkles, acne etc. The purpose of motivation of invention brings into being that guggul being medicinally active ingredients; it could function as a carrier, excipient and synergism also. Following are the some of the observation, which justifies the present study

1. When guggul is rubbed or triturated with water it results in a milky dispersion. Microscopic observation of this dispersion reveals presence of tiny globules, particle and vesicle etc.
2. When drug material, dyes or markers etc. are triturated with guggul in presence of water, the globules, particle or the vesicle formed in the dispersion, take them up. This uptake of is not limited to only lipophilic materials; the hydrophilic materials are taken up as well. This behavior of guggul is analogous to the liposome forming behavior of phospholipids.
3. The entrapped moieties shows much slower dialysis diffusion as compared to their true solution counterparts

So, guggul has been reported to mix properties of liposome, solid lipid particles and multiple emulsions etc.[4,5].Keeping in view, the reported uses of lipids in drug delivery for making liposome's, niosomes, nanoparticles, microemulsion, multiplemulsion and solid lipid nanoparticles, it is proposed to take-up guggul, due to its guggulipid[6] guggulsterones Z&E, resin, gum and volatile oil etc. for investigation as a carrier for the drug delivery because it forms tiny vesicles in contact with water referred to as guggulosomes as like liposome's where is the use of phospholipids.

EXPERIMENTAL

Collection and Identification of Guggul

For present study the material was procured from local market of Mandsaur, M.P., India and authenticated and identified by Dr. S. N. Mishra, Department of Agriculture, Agriculture College, Mandsaur, and M.P., INDIA.

Preparation of Guggulosome

Guggulosomes were prepared by two methods

***Sonication Method*:** Two hundred milligrams of guggul was accurately weighed and mixed with equal quantity of Ibuprofen homogeneously in 10 milliliters of distilled water and volume was adjusted upto 20 ml mark with distilled water, and the resulting suspension was sonicated to give fine vesicles.

***Trituration Method*:** Two hundred milligrams of guggul and equal quantity of Ibuprofen were transferred to a mortar having 10 milliliters of distilled water and triturated. The volume was adjusted upto 20 ml mark with distilled water. Continuous triturating resulted in fine uniform vesicles.

Physical Characterization

Size and Shape: The mean vesicle diameter of the prepared guggul entrapped vesicle was measured using an optical microscope at 100x magnification with the help of a stage micrometer. The shape of vesicle was also observed using optical microscope. The mean vesicle diameters were reported in table 1.

The particle size of the prepared Guggulosomes were determined by optical microscopy using following formula

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$$LeastCount = \frac{N_1}{N_2} \times 0.01mm \quad \{1\}$$

Where N_1 = division of eyepiece
N_2 = division of stage micrometer

0.01mm = 1 division of stage micrometer

Average diameter was obtained by using following formula:

$$\text{Average diameter} = \frac{\sum nd}{\sum n} \times leastcount \quad \{2\}$$

n = number of particles
d = mean diameter of particle

Transmission Electron Microscopy (Tem)

Samples were coated with Cu grid, stained with 2% phosphotungstic acid (PTA) solution and dried under room temperature under different systemic process in TEM (Morgagni 268 D, FEI Netherlands). Guggulosomes were observed at 28,000 magnifications by applying 80Kv energy. Fig.2

Determination of Guggulosomes Concentration

Appropriately diluted sample (1:100) was observed under microscope in each small square of the cell and counted. For present investigation four groups of 16 small squares (total 64 small squares) were selected. Following formula was used to determine numbers of globules per cubic mm were given in table no.1

$$\text{No of globules/mm}^3 = \frac{\text{No. of Globules} \times Dilution \times 4000}{\text{No of small squares counted}} \quad \{3\}$$

In Vitro Drug Release from Ibuprofen Loaded Guggulosomes

In vitro studies were conducted by transferring twenty ml of each Ibuprofen loaded guggulosomes (prepared by sonication or trituration method) to diffusion cell suspended in 60 ml of phosphate buffer (pH 7.4) at 37±1^{0}C, the samples (2ml) were withdrawn periodically and analyzed spectrophotometrically using phosphate buffer pH 7.4 as blank at 221nm. The volume of dissolution medium was replaced with phosphate buffer pH 7.4 after each withdrawn to maintain the final volume of receiver compartment to 60 ml.

Aliquots of sample were withdrawn at predetermined time interval for every 1 h up to10 h then after 24 hr. the drug content in the withdrawn samples was estimated at nm and cumulative % of drug released was estimated and plotted against time (t). This study was also performed for guggul and true solution of Ibuprofen.

Entrapment Efficiency of Ibuprofen Loaded Guggulosome

Percent drug entrapment of drug-loaded guggulosome was determined by separating unentraped drug from guggulosomes by centrifugation at 10000 rpm for 3 hours with equal quantity of alcohol in centrifuge and the supernatant was analyzed for unentraped drug content spectrophotometrically. Encapsulation efficiency of guggul-entrapped vesicle was determined by estimating the amount of free drug and deducting it from the total drug added.

Anti-Inflammatory Activity of Guggulosome by Carrageenan Induced Rat Paw Oedema Method

Healthy Albino rats weighing 150-200 gms of either sex were used for acute toxicity study and anti-inflammatory activity. The animals were housed in polypropylene cages and maintained at 27^{0}C±2^{0}C and 65±10% RH under 12 hour's light/dark cycle. The animals were fed with wet gram and water ad libitum. Animal ethical clearance for performing the experiments on animals was obtained from the institutional Animal Ethical Committee (IAEC)

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The Guggulosomes were evaluated for the anti-inflammatory activity by carrageenan induced rat paw oedema method. Twenty four albino rats of either sex (225-250 gm) were fasted for 24 hr but had free access to water. Rats were divided into four groups of six animals each. First group served as a control, group II served as standard (received Ibuprofen 100mg/kg) and group III & IV were treated with guggul (100mg/kg), Guggulosomes (100mg/kg) respectively.

After 30min. a sub plantar injection of 0.1ml of 1% carrageenan was administered in the left hind paw to all groups of animals. The paw volume was measured with the help of Plethysmograph at 0 hour before carrageen administration and 1, 2, 3, 4 ,5 up to 24 h(fig.4).

The percentage inhibition of Oedema was calculated using following formula.

$$\text{Percentage inhibition of oedema} = \frac{Vc\text{-}Vt}{Vc} \times 100 \quad \{4\}$$

Where, Vt = mean paw volume of test group.

Vc = mean paw volume of control group.

Statistical Analysis

Results expressed as mean ± S.E. were evaluated by students't' test. Values of $p<0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

Guggulosomes had a mean ± standard error of the mean (SEM) particle size of 0.257 ± 0.046 µm using optical microscopy whereas guggulosomes entrapped with Ibuprofen had particle size between 0.1µm to 2.0µm using TEM and distribution of particles was good.

Figure 1

Vesicles of Guggulusome determined by optical microscopy

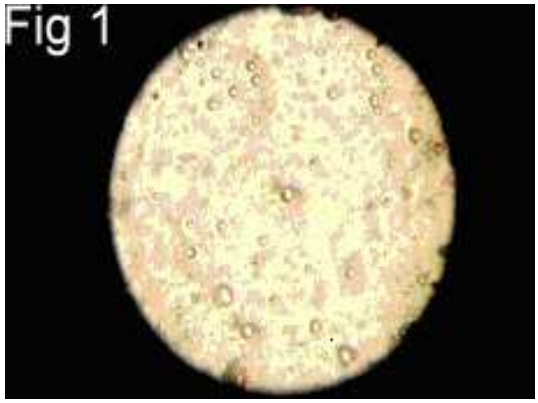
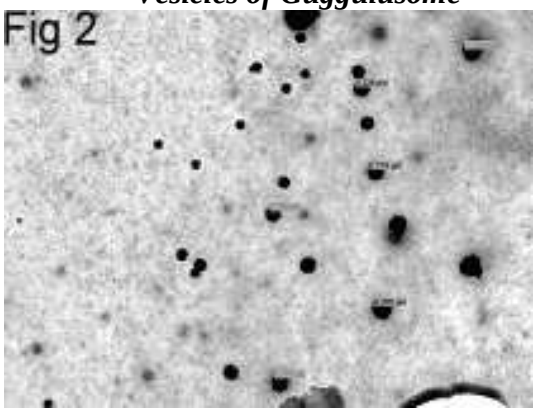


Figure 2

Vesicles of Guggulusome



Vesicles of Guggulusome entrapping with Ibuprofen, prepared by bath sonication method by TEM

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More number of guggulosomes/mm^3 was found using bath sonication methods when compared with trituration method (Table 1)

Table 1

Number of guggulosomes/ mm^3 in different preparation method

S. No.	Preparation Method	Number of small squares	No of guggulosomes	Dilution 100 times of 1 ml (.01)	No. of guggulosomes /mm^3
1.	Bath Sonication	64	82	.01	52
2.	Trituration	64	61	.01	39

Bath sonication method resulted good percent drug entrapment (PDE) with 26.3%± 0.5% of Ibuprofen in guggulosomes while trituration methods resulted in poor PDE of 23.3% ± 0.3 % of the drug. These results indicate that bath sonication method is more effective in terms of entrapment efficiency as well as in terms of average number of particle/mm^3 when compared to trituration method. Bath sonication method is considered better method as this resulted in preparation of guggulosomes with smaller size and more PDE than trituration method. Sonification is high-energy method with ultrasonic irradiation and still remains the method most widely used for producing small vesicle our findings also suggested the same.

In vitro behavior of Ibuprofen loaded guggulosomes prepared by bath sonication was compared with guggulosomes prepared by trituration method. Guggulosomes prepared by bath sonication released 49 % ±0.6 % of Ibuprofen while trituration method released 60 % ± 0.6 after nine hours (Fig 2).This shows that guggulosomes having sustained action.

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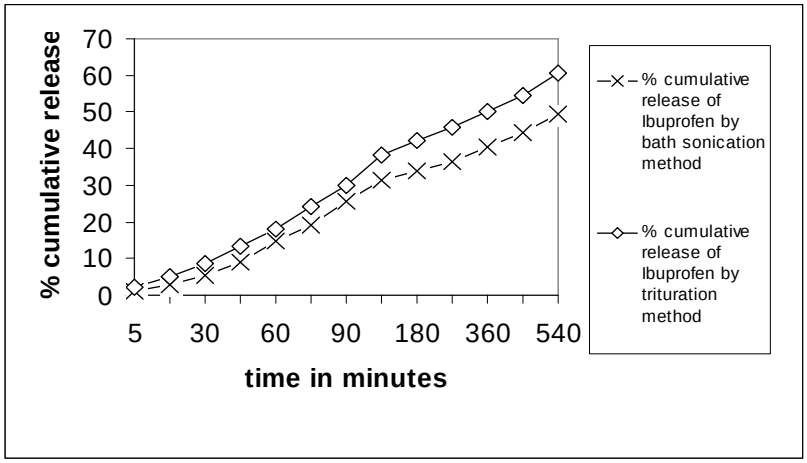
Figure 3
In vitro releases from IP loaded Guggulosomes



Guggulusome is having more efficacy than Ibuprofen and guggul. There is significant increase in efficacy of Ibuprofen due to guggul. The percentage inhibition of inflammation due to Guggulusome increases gradually from 40 to 59 percentages within 4 hours and after the 5 hours the percentage inhibition by Guggulosomes is maintained at 65 %. In case of Ibuprofen the percentage inhibition increases from 38 to 55% in first 4 hours, but at the end of 5 hours it decrease to 47% and in case of Guggul the percentage inhibition increase to 42 %. Guggulusome showed significant anti-inflammatory activity at 5 hours against carrageenan injection, suggesting that the It have sustained and synergistic action.

Figure 4
Percentage inhibition in rat paw oedema method

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Table 4.

Anti-inflammatory activity of Ibuprofen loaded guggulusome by carrageenan induced rat paw oedema method

Treatment	Dose	Volume of oedema (ml) and percentage inhibition at different time interval								
		0hr.	1hr.	% Inh.	2hr.	% Inh	3hr.	% Inh.	4hr.	% Inh.
Control	Saline 5ml/kg	0.311± 0.0054	0.491± 0.0074		0.596± 0.0084		0.891± 0.0087		0.928± 0.0065	
Ibuprofen	100mg/kg	0.271± 0.0047	0.303± .0061***	38.30	0.32± .0068***	46.31	0.38± .0044***	57.37	0.411± .0030***	55.66
Guggul	100mg/kg	0.275± 0.0076	0.333± .0061***	32.26	0.385± .0056***	35.41	0.476± .0049***	46.54	0.515± .0067***	44.52
Guggulusome	200mg/kg	0.29± 0.0044	0.293± .0055***	40.39	0.331± .0047***	44.37	0.36± .0063***	49.62	0.376± .0049***	59.43

Values are mean±SEM (n=6) *** $p<0.001$ vs Control*

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REFERENCES

[1] Alain Rolland. *Pharmaceutical particulate carrier*, Therapeutic application.1st ed. USA, Marcel Dekker,1993,61.

[2] Vyas SP, Dixit VK. *Advanced in Liposomal therapeutics*, 1st ed. New Delhi, CBS Publishers and Distributors,2001

[3] Indian Herbal Pharmacoepia. Revised new edition. Mumbai, Indian drug manufactures association,2002,134-143,

[4] Gupta V B, "Drug Delivery and allied Applications of Guggul" *Indian Patent, Application No. 1715/DEL/2004.*

[5] Jain A, Gupta VB. Chemistry and Pharmacological profile of guggul- a review. *Indian Journal of Traditional Knowledge:5,478-483(2006)*

[6] Derksen J T, Baldeschwieler J D, Scherphof G L. In vivo stability of ester- and ether-linked phospholipid-containing liposomes as measured by perturbed angular correlation spectroscopy *Proc. Natl Acad Sci* , 85(24): 9768–9772, (1988)