Phospholipon 90G Based SLMs Loaded with Ibuprofen: An Oral Anti-inflammatory and Gastrointestinal Sparing Evaluation in Rats

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Abstract.- To formulate and evaluate solid lipid microparticles (SLMs) intended for oral administration of ibuprofen. Ibuprofen-loaded solid lipid microparticles (SLMs) were prepared using hot emulsification method. Characterizations based on particles size and morphology, particles surface charges and stability and encapsulation efficiency (EE%) were carried out on the SLMs. I*n vitro* release of Ibuprofen was performed in phosphate buffer while *in vivo* anti-inflammatory activity and GI sparing effect were carried out in rats. Maximum encapsulation efficiency (EE%) of 89±0.2, 84±0.1, and 93±0.4 for A1-A3, while 81±0.0, 84±0.3 and 94±0.1 were obtained for B1-B3, respectively. Stable, spherical and smooth SLMs of size range 21.1±0.2 µm to 34.2±1.4 µm were produced. The release of ibuprofen in phosphate buffer varied widely with the lipid contents. Moreover, significant ($p<0.05$) anti-inflammatory activity of 65.9, 55.9 and 85.2 % for A1-A3 and 51.3, 65.1, 72.1% for B1-B3 within 6 h respectively were observed. Maximum gastrointestinal (GI) protection of 98, 94, 72 and 71% were observed in batch A3, B3, A1and B1 as compared to 62, 69 and 10 % observed in A2, B2 and the conventional tablet. Thus, SLMs-based on P90G and Beeswax would likely offer a reliable means of delivering ibuprofen orally and prevent GI side effect.

Key words: Ibuprofen, SRMS, SLMS, anti-inflammatory.

INTRODUCTION

Solid lipid microparticles (SLMs) were developed recently and have so far been considered a promising drug carrier system, especially with a view to giving the incorporated active substance a sustained-release profile (Pouton, 2006). Compared with other carriers such as liposomes and microparticles that have been studied for controlled release of incorporated drug, SLMs combine several of those carriers' advantages. SLMs have been found to be physiologically and physicochemically stable and devoid of organic chemical in the preparation (Attama and Muller-Goymann, 2007). Furthermore, other positive attributes of these drug delivery platforms include its excellent stability, ease of preparation and scale-up, and high encapsulation efficiency for lipophilic compounds (Mehnret and Mider, 2001). Therefore, in view of these excellent features of SLMs, investigators the world over find them to be an excellent alternative to other carrier systems.

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID). It is the first member of the propionic acid class of NSAIDs to come into general use (Brunton *et al*., 2010). Ibuprofen has anti-inflammatory, analgesic, and antipyretic activities, and is used for relief of symptoms of arthritis and fever, as an analgesic for pain, especially where there is an inflammatory component, and for dysmenorrhea (Hersh *et al.*, 2000). As a cyclo-oxygenase (COX) inhibitor, it alters platelet function and prolongs bleeding time (Olive, 2006). The healing properties, side effects, and efficacy of ibuprofen have been extensively studied (Jorgensen *et al*., 2006). However, Ibuprofen is rapidly biotransformed, with a serum half-life of about 1.5-2 h that leads to a short duration of action. In order to overcome the shortcoming, multiple intakes of ibuprofen are required to maintain the effective concentration in human bodies which potentially lead to the occurrences of some side effects (Park *et al.,* 2000). A possible approach to resolve this problem is the application of SLM carrying ibuprofen based on solidified reverse micellar solutions (SRMS) (Casadei *et al*., 2006). In other words, to produce different pharmaceutical formulations which ensure

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the satisfaction of the specific needs related to side effect, poor absorption and short half-life, solid lipid microparticles SLM needs to be made by a fusion method. Solid lipid microparticles (SLMs) based on solidified reverse micellar solutions have successfully been employed to achieve controlled release of incorporated drugs. However, mixtures of Beeswax and P90G as solidified reverse micellar solutions (SRMS142) have widely been employed in drug delivery such as gentamicin (Mumuni and Esimone, 2012).

Consequently, solidified reverse micellar solution (SRMS) - based SLMs consisting of Phospholipon 90G and Beeswax could be a potential carrier system for oral delivery of ibuprofen to solve its gastrointestinal side effect, short half-life and absorption problems. The aim of this study was to formulate ibuprofen-loaded SLMs based on SRMS, a conjugate lipid matrix mixture of Beeswax and Phospholipon 90G, characterize the SLMs and evaluate the anti-inflammatory and gastrointestinal protective efficiency of the delivery system in rat model.

MATERIALS AND METHODS

Phospholipon® 90G (P90G) (Phospholipids GmbH, Germany) is a purified, deoiled, and granulated soy lecithin with phosphatidylcholine content of at least 90%. Beeswax was obtained from (Lavan Chemical Ltd, Enugu, Nigeria). Poloxamer 188® (BASF, Germany), Ibuprofen (Spectrum, USA), polyethylene glycol 4000 (Cary Roth, Germany), monobasic potassium phosphate, sodium hydroxide and concentrated hydrochloric acid (BDH, England), distilled water (Lion water, University of Nigeria Nsukka). Other reagents were of analytical grade and used without further purification.

Formulation of the lipid matrices

The lipid matrices consisting of 1:1, 1:2 and 2:1 mixtures, respectively, of Phospholipon® 90G and Beeswax were prepared by fusion (Friedrich and Mu̇ller-Goymann, 2004). Briefly, the lipids were weighed using Adventure® analytical balance (Ohaus, China), melted together on a hot plate at 70°C and stirred using a magnetic stirrer (SR1 UM

52188, Remi Equip., India), until solidification to get SRMS.

Formulation of SLMs

The melt homogenization technique (Pradeep, 2011) was adopted. In each case, the lipid matrix was melted at 70°C, and the aqueous phase containing Poloxamer 188 at the same temperature was added to the molten lipid matrix with gentle stirring with a magnetic stirrer (SR 1 UM 52188, Remi Equip., India), and the mixture was further dispersed with a homogenizer (T 18 digital Ultra-Turrax®; IKA, Staufen, Germany) at 8000 rpm for 5 min to produce the hot primary emulsion. The SLMs suspension obtained after cooling at room temperature was then lyophilized using a freeze-dryer (Amsco/Finn-Aqua® Lyovac GT3, Germany) in order to get water-free SLMs. By adding increasing concentrations of ibuprofen (200 and 400 mg) to the SRMS 1:1, 1:2 and 2:1 and following the above-mentioned procedure, ibuprofen- loaded SLMs (batches A1–A3 and B1–B3) were obtained. SLMs containing no drug (unloaded SLMs) which served as negative control were similarly formulated and labeled as C1–C3. The formulation compositions are shown in Table I.

Determination of percentage yield

The water-free SLMs from each lipid matrix were weighed to obtain the yield of SLMs formulated per batch. The percentage (%) yield was then calculated using the formula:

$$\% \text{ yield} = \frac{W_1}{W_2 + W_3} \times 100 \quad \text{Eqn 1}$$

where W_1 is the weight of the SLMs formulated (g), W_2 the weight of the drug added (g) and W_3 the weight of the lipid and P-188 (g).

Characterisation of solid lipid microparticles

The particle size of the SLMs was determined by photon correlation spectroscopy using a Zetasizer nano (ZEN 3600, Malvern, UK). Measurements were carried out at 25°C at a light-scattering detection angle of 90°C. The mean particle size and polydispersity index were determined. The zeta potential was similarly determined using a Zetasizer by phase analysis light scattering (PALS).

Release of Ibuprofen from SLMs formulation

The drug release study from SLMs was conducted using the method described below. A 50 mg quantity of drug (Ibuprofen) loaded SLMs were added to 250 ml phosphate buffer (pH 7.2) in a conical flask and incubated for 37°C under magnetic stirring at 250 rpm, at different predetermined time intervals, 1.5 ml volumes were withdrawn and replaced with equal volume of the dissolution medium. The ibuprofen content was determined using a UV spectrophotometer (Shimadzu, 1601, Japan) at 221 nm. Drug release data was analyzed using four different kinetic models.

Animals

All the animal experiments in these studies were conducted according to guidelines established by the Institutional Animal Care and Use Committee of University of Nigeria Nsukka, and adhered to the European Community guidelines for the use of experimental animals (86/609/EEC). Albino rats of either sex (Biochemistry Laboratories, University of Nigeria, Nsukka), which weighed 210 to 225g were housed in an animal care facility maintained at 22±2°C on a 12-h light/dark cycle with unrestricted access to food and water. Before the day of experiment, the Wistar rats were fasted overnight with free access to water.

Anti-inflammatory activity

Eight groups of four rats per group were involved in the investigation. The rat in group 1 received blank SLMs (C1 without ibuprofen, 2 ml p.o). The rat in group 2 received distilled water (DW) only (2 ml p.o) and served as negative control (NC), while the commercial sample (Ibumol®) was given to group 3 and served as positive control (PC). Then, the rest rat groups 4, 5, 6 received SLMs A1-A3 and groups 7, 8 and 9 received SLMs B1-B3 of Ibuprofen- loaded SLMs, respectively, with the aid of an intragastric tube according to their body weight. One hour before the administration of test agents, a 0.2 % of γ-carrageenan solution was subcutaneously injected into the right hind paws. The paw volume was measured before drug administration and after the carrageenan injection, using a digital plethysmometer. The percentage inhibition of the edema formation was calculated as follows:

$$The\ percentage = \frac{Control\ group - Test\ group}{Control\ group} \times 100 \quad \text{-- Eqn 2}$$

Evaluation of GI side effect as a function of ulcer induction

The formulations were tested for GI sparing effect as a function of ulcer induction in a rats model. Briefly, batches A1-A3 were administered to groups 1, 2 and 3 and (B_1-B_3) to 4, 5 and 6 respectively, at a dose of 50 mg/kg per oral for seven days. Rats were subjected to midline abdominal incision according to our previous method (Momoh and Adikwu, 2008), abdomen was opened and stomach was removed after ligating both esophageal and pylorus end. Incision was made in the stomach along its greater curvature; mucosal surface was exposed and washed with normal saline. It was then stretched and pinned on cork board. Mucosal surface was then examined for erosions and ulcerations. Ulcer index was calculated using an established standard method (Kumar *et al.*, 2004).

Stability study

Stability study of selected SLMs dispersions was carried out for 8 months. The SLMs were packed in well-sealed amber glass bottles at 4°C. Samples were taken every two months and analyzed for EE% and particle size. In addition, visually inspection was also carried out).

Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean SD. ANOVA and Student's t-test were performed on the data sets generated using SPSS version 14. Differences were considered significant p<0.05.

RESULTS

In this study, different lipid combinations were used in the preparation of SLMs. Results of the particle size analysis of the different SLMs are shown in Table II. The particles sizes of the SLMs ranges from 21–34 µm with batch A1 have the least particle size, while batch A2 showed the highest particle size. The polydispersible index (PD1) were

Table I.- **Quantities of materials used for SLMs formulation.**

Batch	P90H : BW	P-188 (g)	IBRU (mg)	PEG-4000 (g)	Distilled water, q.s (g).
A1	1 : 1	2.0	400	1.0	100
A2	1 : 2	2.0	400	1.0	100
A3	2 : 1	2.0	400	1.0	100
B1	1 : 1	2.0	200	1.0	100
B2	1 : 2	2.0	200	1.0	100
B3	2 : 1	2.0	200	1.0	100
C1	1 :1	2.0	-	1.0	100
C2	1 : 2	2.0	-	1.0	100
C3	2 : 1	2.0	-	1.0	100

Table II.- **Characterization of the SLMs.**

Batch	P90H:BW	PDI	ZP	DL	% Yield	Particle size (µm) ± SD		(%) Encapsulation efficiency	
						1 week of preparation	After 8 months of storage	1 week of preparation	After 8 months of storage
A1	1 : 1	0.745	-43.0	34±0.4	91.0	21.1±0.2	46.4±0.2	89±0.2	86±0.2
A2	1 : 2	0.885	-48.7	23±0.1	93.9	34.2±1.4	69.2±1.5	84±0.1	82±0.1
A3	2 : 1	1.000	-53.1	30±0.2	96.6	28.8±0.21	38.2±1.2	93±0.4	91±0.4
B1	1 : 1	1.000	-59.4	56±0.6	88.3	24.1±0.1	34.1±1.1	81±0.0	81±0.0
B2	1 : 2	1.000	-53.2	52±0.1	89.4	29.6±0.11	76.1±2.1	84±0.3	81±0.3
B3	2 : 1	0.885	-48.7	23±0.1	94.2	24.2±1.4	44.2±1.4	94±0.1	90±0.1

PDI, polydispersibility index; ZP, zeta potential; Mob, mobility; DL, drug loading; EE, encapsulation efficiency.

Table III.- **Anti-inflammatory activity and the GI effect of ibu-loaded SLMs.**

Batch	P90H:BW	% Anti-inflammatory inhibition (Time h)					GIT sparing effect		
		1	2	3	4	6	Mean Ulcer	Index ± SD	% Protection
A1	1 : 1	15.4	23.2	35.8	63.0	65.9	1.03±11		72
A2	1 : 2	12.2	31.4	33.3	49.1	55.9	1.21±02	66	
A3	2 : 1	26.1	30.9	35.2	63.2	85.2	0.98±18		98
B1	1 : 1	11.6	21.1	25.6	49.0	51.3	1.15±13		71
B2	1 : 2	10.0	35.1	63.2	49.0	72.1	1.61±10	69	
B3	2 : 1	8.5	32.8	53.7	65.1		1.02±03	94	
Ibunol	(PC)	14	25.3	35.6	51.6	47.21	5.34±11	-	10
C1	(PC)	-	-	-	-	-	-	-	-
DW	-	-	-	-	-	-	-	-	-

Values are mean ± S.E.M. (n = 5), Significant as compared to control $P < 0.001$

between 0.745-1.00, Zeta potential (ζ) ZP were negative and the encapsulation efficiency were all high.

In vitro *release of Ibuprofen from SLMs*

The in vitro release profiles of ibuprofen in phosphate buffer indicate very significant release of ibuprofen from all batches of the formulation as shown in Figure 1. In batch A formulations, sub-batch A3 gave a maximum cumulative release of 91%, while sub-batch A1 gave the least (maximum cumulative release of 58%). Additionally, in batch B formulations, sub-batch B3 exhibited the highest amount (*i.e.* 88%) of the drug, while sub-batch B1 gave the least amount (59 % of the drug).
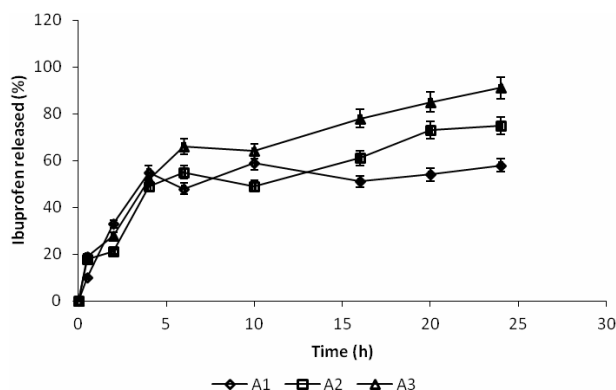
Fig. 1. *In vitro* release profile of ibuprofen from SLMs formulated with: A1, SLMs 1:1; A2, SLMs 1:2 and A3, SLMs 2:1 in phosphate buffer system. A1–A3 contains 400 mg of ibuprofen.

Kinetic analysis of in vitro *release profiles*

Different mathematical models were used to describe the kinetics of ibuprofen release from the SLMs. The criterion for selecting the most appropriate model was the goodness-of-fit test.

The results revealed that Higuchi and Kosemeyer models can serve to explain the cumulative release profile of Ibuprofen release from the SLM formulations.

Table III shows the results of the anti-inflammatory and GI sparing effect. The percent inhibition of inflammation was evident in all the doses administered and the GI protection was high in batches with higher levels of P90G content.

DISCUSSION

The percentage yield (Table III) of the sub-batch A3 was higher compared to other batches in the group, the same applied to the sub-batch B3, but when batch A3 and B3 were compared, it is obvious that the yield in A3 is slightly higher than B3. This may be attributed to the quantity of drug added to the matrix; it is an indication that SLMs were able to accommodate more drug, which could be due to the structural configuration of the lipid core. Additionally, encapsulation efficiencies were high and increase concomitantly with increase in the drug concentration, the drug loading also followed the same pattern as shown in Table II.

The zeta potential (Table II) are all negative and range from (-43 to -59 mV). The zeta potential is a surrogate marker of the stability of the formulated SLMs. It is noted that the charges on the surfaces of the particles play an important role in establishing the stability and pharmacokinetics of pharmaceutical preparations. Furthermore, it could be shown that particles with a positive or neutral zeta potential had a slower clearance from the blood via macrophages than negatively charged ones in terms of peptide (del Pozo-Rodriguez *et al*., 2010). More so, positively charged particles interact with the negatively charged surface area of the mucosal wall easily than the negatively charged particles, however, negatively charged SLMs particles possess electrostatic tendency by means of which the incorporated drug is adsorbed onto the surface of the SLMs leading to burst effect (del Pozo-Rodriguez *et al*., 2010). PEG been a non-ionic surfactant used in this study may promotes steric repulsion of the particles and as a consequence dominates the attraction force, thereby culminating in enhanced product stability. This may be so, because of the hydrophilic moiety of polyethylene glycol chains (PEG) which shield the lipid core of the microparticles isolating them from their aqueous surroundings and preventing the efflux of the drug to the surfaces. The net effect of this shielding action is the observed sustained release of the incorporated Ibuprofen in the microparticles studied. In addition, the (PEG) hydrophilic layer is also implicated in bringing about the slow plasma clearance of the drug from the system which is the main target of control release formulation. Results obtained suggest that Ibuprofen- loaded SLMs could be an appropriate delivery system, as it eliminates multiple dosing associated with the conventional immediate release formulations.

The FT-IR results show no interactions on comparison of IR spectra of pure ibuprofen drug, phospholipids, beeswax and the SLMs loaded drug representative. The results obtained showed no interaction between the drug and the polymer (Figures not shown).

The in vitro release profiles of Ibuprofen indicate very significant release of the drug in all the batches of the formulation (Figs. 1, 2). The highest release was obtained in Batches A1 and B1, while

A2, A3, B2 and B3 gave lower cumulative drug release. This could be ascribed to, most probably, retardation owing to association of the active ingredient to the phospholipid since A3 and B3 contain more P90G, it is posited that the P90G shields the surface area of the lipid which causes a delay in the diffusion of the drug out of the core lipids. While batches A2 and B2 may be due to drug enclosed in the core of the lipid (Beeswax) that was more in the batch. The results here were in agreement with the findings of previous workers (Raffin *et al.*, 2007), who employed P90G as a retarding agent.
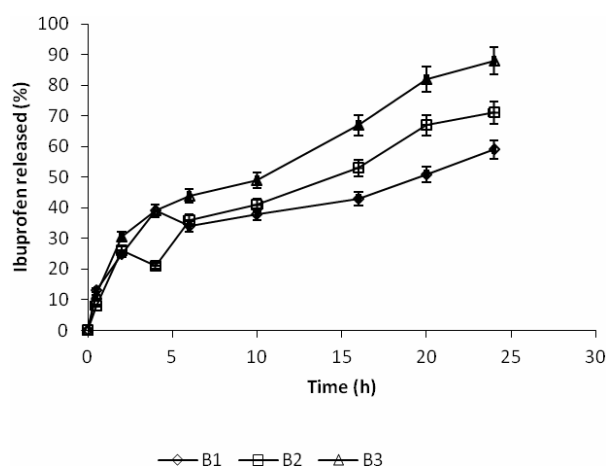

Fig. 2. *In vitro* release profile of ibuprofen from SLMs formulated with SLMs 1:1; B1, SLMs 1:2; B2 and B3, SLMs 2:1 in phosphate buffer system. B1–B3 contains 200 mg of ibuprofen.

The kinetic analysis of the in vitro release profiles revealed that the release kinetics of IBU loaded SLMs tend to follow the Higuchi release kinetics in all the formulation (r^2 = 0.9280, 0.9903, 0.9452 and 0.9284 for A1, A2, A3 and B1), respectively. This indicates that the release of drug is proportional to the square root of time. Additionally, the rate of release can be increased by increasing the drug's solubility in the lipid matrix and vice versa. However, sub-batches B2 (1:2) and B3 (1:2) followed Higuchi and Hixson–Crowell models of release (r^2= 0.9258, 0.7356 and 0.7113, 0.8335), respectively. This implies that IBU-loaded

SLMs undergo a change in surface area as IBU dissolves and diffuses out of the hydrated or solvated SLMs in these batches. Generally, since most of the formulations followed the Higuchi square root kinetics of release model indicating a diffusion controlled release process, as expected from a heterogeneous matrix system (Attama *et al.*, 2003; Friedrich and Muller-Goymann, 2003), it implies that the smooth surface of the P90G-based IBU-loaded SLMs have enough pores and channels to permit controlled drug release.

Table III and Figure 2 show the anti-inflammatory and GIT sparing effect of SLM formulations and standard drug as compared with controls at different time intervals following carrageenan-induced paw edema. The observed inhibition of edema by the test SLM formulations indicates that the ibuprofen-loaded SLMs prepared with the various ratio of P90G and beeswax produced anti-inflammatory lowering effect much higher than those of either pure drug dispersed in water alone or ibuprofen marketed samples. The high level of anti-inflammatory inhibition observed with the formulations employed in this study indicates that there may be synergism between the carriers in permeating the mucosa wall for effective absorption within the GIT. The SLMs prepared with one part beeswax and three parts P90G produced maximum anti-inflammatory reduction 3 h after oral administration that was greater than that of marketed samples. Trends observed in Table III, Batch A (1:1) also shows a slight reduction in inflammatory level after oral administration of SLMs. However, it is quite obvious that high lipid content in this batch may not necessary enhance absorption of the incorporated drug into the system contrary to what is observed in the *in vitro* release thus, the reduction in the inflammatory level is not in congruence with the result of the release study. GIT sparing results revealed that IBU loaded SLMs consisting of BW and P90G in a ratio of 1:3 were well tolerated by rats at the concentrations tested. There was a significant difference observed between the formulation with high P90G ratio to that of Beeswax (2:1) and other group with respect to erosion of the GIT. However, compared to controls, the positive control group showed that ulcer was induced along with severe hemorrhage. The batch

that contain (1:2) of P90G and BW show a similar trend of ulcer formation, but comparatively less severe than that of the positive control. There was no such observation in the negative control group (animals fed with distilled water alone). This may be due to the high content of P90G which is the varying factor from other groups. Studies have shown that P90G has the tendency to maintain GI integrity (Kuo and Chen, 2007). The exact mechanism through which this protection of the GIT is effected is unclear, the assumed mechanisms are that P90G prevents the increase of the permeability of GIT that is associated with NSAIDs, thereby improving the barrier properties of the gastric mucosa and reducing the risk of gastric hemorrhage. In addition, Prostaglandins are known for their cell-protective effect, and the protective mechanism postulated here also suggests that prostaglandin synthesis is triggered by P90Gs as its precursor in the cells of the gastric mucosa. Other researchers proposed a similar mechanism in his study on the aspirin delivery using unsaturated Phospholipon 90G (del Pozo-Rodriguez *et al.*, 2010; Rafin *et al.*, 2007).

Stability of pharmaceuticals preparations is a key factor in Good Manufacturer Practice (GMP) and also a critical determinant of the therapeutic efficacy of formulations in clinical practice. Many lipid preparations have been reported to exhibit varying physical stability at a low temperature, despite it highly ordered crystal packing (Kuo and Chen, 2007). In the current study, SLM dispersions were stored in closed bottles at $4^{o}C$ in an attempt to investigate the physical stability of SLMs in the liquid state. Particle size range and EE% data are shown in Table II. Visual inspection revealed no aggregation or separation of SLMs over the study period. Results revealed that SLMs maintained their particle size, indicating good physical stability of the particles. In addition, EE values were also unchanged; this suggests that no drug expulsion occurs from the formulations elaborated for the period of the study. One clear trend is the beneficial role of co-surfactants. Solid lipid microparticles stabilized by surfactant mixtures, such as PEG or Poloxamer 188, resulted in more stable, smaller particle sizes than formulations of the same lipid and a single surfactant (Marengo *et al.*, 2007).

CONCLUSIONS

Over the past two decades, significant advances have been made in understanding the pathogenesis of NSAID-induced GI injury. The identification of key intracellular events in the development of ulcers after NSAID administration has provided important signs of the Achilles heel for designing contemporary Trojan horses such as novel drug delivery systems like the (SLMs based on P90G and Beeswax) loaded anti-inflammatory drugs with reduced toxicity. This shows that the use of phospholipids as a carrier can reduce the side effects of orally administered active ingredients on the gastro-intestinal tract without affecting the therapeutic action of the NSAIDs.

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DECLARATION OF INTEREST

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