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Anti-Inflammatory and Antinociceptive Activity of Herbal Lipospheres of *Pentaclethra macrophylla* (Fabaceae) Stem Bark Extract

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Abstract: Purpose: Inflammation of various degrees is common among humans. There are associated side effects with orthodox delivery systems and anti-inflammatory agents; hence, the study investigated the characteristics of herbal lipospheres and the anti-inflammatory potency of the lipospheres formulated from *Pentaclethra macrophylla* with the view to having a drug with a better delivery system and lesser side effects. Methods: Herbal lipospheres were formulated using solidified reverse micellar solutions (SRMS) of P90H and goat fat and characterized for particle size and morphology, pH time dependent analysis, encapsulation efficiency (EE%), and Fourier Transform infrared spectroscopy. The in vitro antinociceptive and anti-inflammatory studies were carried out using membrane stabilization by hypotonicity-induced hemolysis and the determination of anti-platelet aggregatory activity models. The in vivo antinociceptive and anti-inflammatory studies on egg albumin- and formaldehyde-induced arthritis models were conducted. A total white blood cell count and differential blood count were carried out on the rats. Results: The results showed that there was no change in pH for the PM-unloaded lipospheres and 2.5 g of PM-loaded lipospheres from day 1 to day 7, but there was a mild variation in the rest of the formulations. The EE ranged from 35.2% to 94%, increasing according to the drug concentration. The photomicrographs of the lipospheres showed that the particles were spherical in shape. The particle sizes were within the acceptable range for lipospheres. FTIR showed no interaction. In the arthritis study, PM-loaded lipospheres inhibited edema consistently throughout the duration of observation. Inhibition of the membrane increased steadily with an increase in concentration of PM in the lipospheres and the standard drug. The platelet aggregatory inhibition decreased steadily with an increase in concentration of the PM in the lipospheres as well as the standard. The T50 dose of PM had the highest percentage of WBC, and it decreased as the treatment doses increased from T100 to T200. There were no significant differences among the Neutrophil counts of the different groups. Conclusions: The study, therefore, showed that the methanol extract of *Pentaclethra macrophylla* formed efficient herbal lipospheres with antinociceptive and anti-inflammatory activities.

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1. Introduction

Pentaclethra is a small genus of the Fabaceae family, comprising mainly three species of trees, distributed throughout subtropical and tropical regions of the world [1–4]. The species of the genus Pentaclethra, such as *Pentaclethra macroloba* and *P. eetveldeana,* have been described as medicinal plants with their biological activities assessed [3,4]. *P. macrophylla* is the only member of the genus occurring naturally in the lowlands of West Africa. It is abundantly found in the southern Middle Belt and south eastern Nigeria [5–7], with no varietal characterization [8].

Nearly all parts of the *P. macrophylla* plant are used for various human and animal ailments. The fruits, seeds, stem bark, leaves and roots have been reportedly used in traditional herbal practices in different countries. The stem bark has been credited in Nigeria as antiulcer [9], antidiabetic [10], anti-inflammatory [11], antigonorrhea [12], antimicrobial [13], antinociceptive [14], anticonvulsant and antitussive [12]. In Nigeria, the seeds have been reported as anticancer [15] and abortificient [10]; in Cameroon, it is used for infertility [16]. The root bark is an enema against dysentery, a liniment against itching, a laxative and abortificient as an infusion in both Nigeria and Cameroon [14]. The leaf is used to treat diarrhea, fever, stomach ache and convulsion [8,16,17]. The plant has also many nutritional benefits [18,19]. In Ghana, it has been reported as an anthelmintic for leprosy sores [13], antiepileptic [20], antidiarrheal [17], antipruritic and for wound management [16]. Bergenin is an active constituent of the plant and research indicates that bergenin has multiple biological activities, including anti-inflammatory and immunomodulatory properties [5]. The ethnobotanical evidence indicates that the traditional use of *Pentaclethralmacrophylla* in Nigeria is perhaps related to the plant's anti-inflammatory and antinociceptive properties. However, since the pharmacological properties of *P. macrophylla* have rarely been reported, the use of this plant has continued without solid scientific evidence to support it. The present study was carried out to evaluate the antinociceptive and anti-inflammatory properties of lipospheres prepared with the methanol extract of *P. macrophylla* and administered orally in animal models of pain and inflammation.

In inflammation, the lysis of some cellular component leads to the release of inflammatory mediators [21]. When erythrocytes are exposed to hypotonic medium, heat, methyl salicylate and phenylhydrazine, among others, the lysis of their membrane occurs. It results in the leakage of serum protein and fluids into the tissues instigating inflammation [21]. A membrane stabilization assay of erythrocytes is a widely used tool to investigate the anti-inflammatory potential of a plant extract [21]. Because of its simplicity and economy, researchers prefer to use this model in the preliminary screening of medicinal plant extracts. Therefore, extracts showing membrane stabilization might be very suitable as anti-inflammatory agents [21]. Allopurinol, oxypurinol and tisopurine (xanthine oxidase inhibitors), steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs, are employed in the treatment of arthritis and inflammation in humans. Orthodox drugs are replete with various side effects, hence a continued search for natural remedies that can reduce the accumulation of uric acid and naturally inhibit cyclooxygenase-2 with much lesser side effects are highly needed. Inflammation is commonly found among different strata of humans. As humans age, the metabolism seems to be challenged at different points, uric acid accumulates and pains of various degrees set in naturally, so many resort to different drugs to ameliorate the pains. The level of literacy, economic well-being and shear disregard to caution have led many to abuse different drugs, including NSAIDs. Hence, the study investigated the characteristics of herbal lipospheres and the anti-inflammatory potency of the lipospheres formulated from *Pentaclethra macrophylla* with the view to having a drug with a better delivery system and lesser side effects.

2. Materials and Methods

2.1. Collection and Identification of Pentaclethra macrophylla Stem Bark

The stem bark of *Pentaclethra macrophylla* tree was collected from Ejuona Obukpa Nsukka, Enugu State, Nigeria, in March 2022 and authenticated by a plant taxonomist Mr. Alfred Ozioko of the International Centre for Ethno medicine Development (Inter-CEED) Nsukka, Enugu State, Nigeria. The voucher specimen (PCG/UNN/014/507) was assigned to the sample which was deposited in the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka, Nigeria. The plant material was collected in line with the ethical guide of the University of Nigeria, Nsukka, on herbal research.

2.2. Maceration and Extraction of Plant Material

The fleshy stem bark of *P. macrophylla* was harvested, thoroughly rinsed with distilled water and air-dried at room temperature (28 ± 2 °C). Dried plant portion (2 kg) was mechanically pulverized using a hammer mill (Henan Always Machinery, Zhengzhou, China). Methanol (MeOH, 98%) extraction was carried out according to an earlier method [9] at room temperature for 72 h with vigorous shaking at intervals. The mixture was filtered using a muslin cloth and the resulting filtrate was further filtered through multiple fluted filter papers (Whatman No. 1 filter paper) (Sigma-Aldrich, Johannesburg, South Africa) and the final filtrate concentrated in vacuo at 40 °C to obtain dry crude methanol extract. Extracts were stored in sterile containers until further use.

2.3. Extraction of Goat Fat from Capra hircus

The adipose tissue of freshly slaughtered *Capra hircus* (goat) was obtained from the abattoir in Ikpa market Nsukka and freed of extraneous materials. The fat was extracted from the adipose tissue according to an earlier method with modification [22]. Briefly, the adipose tissue was grated and boiled with half its weight of water on a water bath for 45 min. The molten fat was separated from the aqueous phase using a muslin cloth. The fat was further purified by heating the lipid with a 2% *w*/*w* suspension of activated charcoal and bentonite (1:9) ratio blend at 100 °C for 1 h. Thereafter, the suspension was vacuum filtered using a Buchner funnel. The fat was stored in the refrigerator at 4 °C.

2.4. Preparation of Lipospheres

The lipid matrices were first prepared according to Table 1. Briefly, the solidified reverse micellar solutions (SRMS) of Phospholipon 90H® (GmbH, Köln, Germany) and goat fat corresponded to 1.25% (0.75:0.5), respectively. A 0.5 g quantity of goat fat was carefully weighed into a beaker and melted at 80 °C using a hot water bath. Then, 0.75 g of Phospholipon 90H® was transferred into the melted goat fat and both were stirred continuously with a glass stirrer to ensure adequate mixing until a homogenous transparent liquid was obtained. Consequently, the aqueous phase consisting of Tween 80, sorbitol, sorbic acid and graded concentration of PM stem bark extract (0, 2.5, 5.0, 7.5 and 10 g) were equally heated to the same temperature and later added to the lipid phase. Lipospheres were formulated by high shear homogenization (Ultra-Turrax, T18 basic, IKA, Staufen, Germany) at 18,000 rpm for 5 min. The resultant lipospheres were cooled at room temperature and stored in amber-colored bottles firmly secured for further analysis.

Table 1. Composition of lipospheres.

PM Batches (g)	Goat Fat (g)	Phospholipon 90H (g)	Sorbitol (g)	Sorbic Acid (g)	Tween 80 (g)	Distilled Water (qs) *w*/*w*
0	0.5	0.75	4	0.05	1.5	100
2.5	0.5	0.75	4	0.05	1.5	100
5.0	0.5	0.75	4	0.05	1.5	100
7.5	0.5	0.75	4	0.05	1.5	100
10.0	0.5	0.75	4	0.05	1.5	100

2.5. Characterization of Lipospheres

2.5.1. Determination of Particle Size and Morphology of Lipospheres

Microscopic evaluation and particle size analysis of the lipospheres were performed at ×100 magnification, in which 5 mg of liposphere from each batch was placed on a slide, covered with a cover slip and observed using a compound phase-contrast microscope (Motic B3; Motic, Carlsbad, CA, USA). All digital micrographs were captured using Moticam 2.0 image system (Motic, Carlsbad, CA, USA). Each sample was assessed five times and representative measurements were made for each sample. The particle dimensions (height, width, area and perimeter) were measured in micrometer (μm).

2.5.2. pH—Time Dependent Analysis

After the liposphere preparation, the pH of the batches was determined using a pH meter (Hanna, H198108). The pH analysis and stability of the loaded and unloaded lipospheres were determined in a time-dependent manner (24 h, 7 and 30 days).

2.5.3. Encapsulation Efficiency (EE %)

The entrapment efficiency of the liposphere formulations was calculated by using the indirect method of ultrafiltration using Amicon filter tubes (Germany), which consisted of a filter membrane with a molecular weight cut-off of 10,000 at the base of the sample donor chamber. For this, the liposphere (1 mL aliquot) of undiluted sample was placed in the upper chamber and the sample recovery chamber was fitted below the membrane in the lower compartment. The unit was closed and centrifuged at 2500 rpm for 5 min using a centrifuge (Model 420R Rotina Hettich, Germany) at 25 °C in order to separate microparticles from the unentrapped drug and the free drug in the supernatant was quantified afterwards using HPLC. The encapsulation efficiency was calculated through the formula:

$$EE\ (\%) = \frac{\text{Theoretical drug content} - \text{actual drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

The loading capacity was determined in relation to the total weight of the lipids using the formula:

$$LC\ (\%) = \frac{Wa - Ws}{Wa - Ws + Wl} \times 100 \quad (2)$$

W_a is the weight of the *P. macrophylla* added to the formulation and W_s is the actual amount of *P. macrophylla* encapsulated in the lipospheres. W_l is the weight of the lipid added in the formulation.

2.5.4. HPLC Method

HPLC determination of *P. macrophylla* in methanol was performed using an ASI-100 automated sample injector equipped with UV-vis detectors operating at 274 nm. Samples were chromatographed on a stainless steel C18 reverse phase column (Luna (2) 150 × 4.6 mm) packed with 5 mm particles (Lichrospher1 100 RP-18). For the separations, a gradient of mobile phase A (0.1 v/v % TFA in acetonitrile) and mobile phase B (0.1 v/v % TFA in water) was used. TFA was selected to provide pH 2 so as to have narrow peak shapes. The flow rate was 1.0 mL min^{-1}, the column temperature was 40 °C and the injection volume was 10 μL. The UV detection wavelength was 274 nm. The detector cell temperature was 40 °C. A calibration curve was plotted for the extract in the concentration range of 2–10 mg/mL. A good linear relationship was observed between the concentration of extract and its peak area with a percentage correlation coefficient of 99.37%. The required studies were carried out to estimate the precision and accuracy of the HPLC method.

2.6. FTIR Analysis

The unloaded and loaded liposphere formulations (0, 2.5, 5,7.5 and 10 g) were analyzed for FTIR at spectra range of 4000–500 cm. A 0.4 g of KBr was weighed and ground to powder. Then, 0.001 g of the samples containing *Pentaclethra macrophylla* (2.5, 5, 7.5 and 10 g) was

separately weighed into the ground KBr and both were thoroughly mixed and molded into a disc. The disc was inserted into the sample compartment of the instrument and the IR spectrum was generated (Shimadzu FTIR. Model IR Affinity-1, Kyoto, Japan). The same procedure was repeated for the unloaded (placebo) liposphere.

2.7. In Vitro Anti-Nociceptive and Anti-Inflammatory Study

2.7.1. Assay of Membrane Stabilization by Hypotonicity Induced Hemolysis

The sample batches of the *P. macrophylla*-loaded lipospheres, unloaded lipospheres and acetyl salicylic acid (Aspirin) were dissolved in distilled water (hypotonic solution). A 5 mL hypotonic solution containing graded doses of the lipospheres (100, 200, 400, 600 and 800 µg/mL) were made into duplicate pairs (per dose) of the centrifuge tubes. Then, 5 mL of the isotonic solution containing graded doses of the unloaded and loaded lipospheres (100–800 µg/mL) were prepared and put into duplicate pairs (per dose) of the centrifuge tubes. The control tubes contained 5 mL of the vehicle (distilled water) and 5 mL of 200 µg/mL of Aspirin, respectively. Erythrocyte suspension (0.1 mL) was added to each of the tubes and mixed gently [21]. The mixtures were incubated for 1 h at room temperature (37 °C) and then centrifuged for 5 min at 1300 rpm. Absorbance (OD) of the hemoglobin content of the supernatant was estimated at 540 nm using a UV spectrophotometer (Milton Roy). The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water was 100%.

The percentage inhibition of hemolysis was calculated using the following relation:

$$\% \text{ Inhibition of hemolysis} = 1 - \frac{\text{OD2} - \text{OD1}}{\text{OD3} - \text{OD1}} \times 100 \quad (3)$$

where OD_1 = absorbance of test sample in isotonic solution,
OD_2 = absorbance of test sample in hypotonic solution,
OD_3 = absorbance of control sample in hypotonic solutions.

2.7.2. Determination of Anti-Platelet Aggregatory Activity

This method of antiplatelet activity determination is a modification of Born and Cross [23]. Briefly, 5 mL of fresh blood sample was withdrawn intravenously from healthy volunteers using 5 mL plastic syringes and put into plastic tubes containing 0.01 mL of 1% EDTA as the anticoagulant. The EDTA plastic tubes were centrifuged at 3000 rpm for 10 min. The blood sample was separated and the supernatant was collected, diluted twice using normal saline and then used as platelet-rich plasma [24]. Absorption changes of the platelet rich plasma (PRP) were determined. Then, 0.2 mL of the platelet rich plasma, 0.4 mL of the 2M $CaCl_2$, varying concentrations of the *Pentaclethra macrophylla*-loaded lipospheres (50, 100, 150, 200 mg representing 2.5, 5, 7.5 and 10 g formulations, respectively), unloaded lipospheres and normal saline were incubated. The absorbances of the solutions were measured at 520 nm using UV spectrophotometer (Milton Roy, Houston, Texas, USA). The changes in absorbances of the solutions at 520 nm were taken at time intervals (0, 30, 60, 90 and 120 s).

2.8. In Vivo Antinociceptive and Anti-Inflammatory Study

The animal experimental protocols were in accordance with the guidelines for conducting animal experiments stipulated by our Institution's Animal Ethics Committee and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of 24 November 1986 (86/609/EEC). Briefly, the rats were divided into groups and acclimatized to the laboratory environment a week before the study.

2.8.1. Egg Albumin Induced Rat Paw Edema Inflammatory Model

Fresh egg albumen-induced rat hind paw oedema was used as model for acute inflammation. Some 28 adult Wistar albino rats of either sexes were weighed and divided

into 7 groups of 4 rats each. The animals were allowed to acclimatize to the environment for 7 days before the experiment. The animals were then fasted and deprived of water for 18 h before the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in edematous response [24]. Immediately after the deprivation, their right hind paw volumes were taken at time zero (t = 0) volume (V = V_0) using a Vernier caliper. The first group of four animals were induced with edema but did not receive any treatment, the second group received 20 mg/kg of Diclofenac (positive control group), the third group received 50 mg/kg of blank liposphere containing no drug, while the fourth to seventh groups received different doses (50, 100,150 and 200 mg/kg body weight corresponding to 2.5, 5, 7.5 and 10 g) of the *P. macrophylla*-loaded lipospheres, respectively. One hour after the administration of the test substances, 0.1 mL of undiluted fresh egg albumin was injected into the sub-plantar region of the right hind paw of the rats to induce acute inflammation. After the egg albumin administration, the paw volumes were measured using a vernier caliper at 0, 1, 2, 3, 4 and 5 h. Formation of oedema was then assessed in terms of the difference in the zero time paw edema volume of the injected paw and its paw edema volume at different times after egg albumin injection of the right hind paw. Average inflammation, % inflammation and % inhibition of inflammation were calculated for each dose of the loaded liposphere containing *P. macrophylla* using the following relationships:

$$\text{Average inflammation} = (V_T - V_0) \quad (4)$$

where V_0 = Volume of oedema at time zero (initial time); V_T = Volume of oedema at time T (0.5, 1, 2, 3, 4 and 5 h)

$$\%\ \text{inflammation} = \frac{\text{Average inflammation of treated groups at time T}}{\text{Average inflammation of control at time T}} \times 100 \quad (5)$$

$$\text{Then, }\%\ \text{inflammation} = \frac{(\text{VT–V0 }) \text{ groups } 1,2,3,4,5}{(\text{VT} - \text{V0 }) \text{ control}} \times 100 \quad (6)$$

$$\%\ \text{inhibition} = \text{Average inflammation of control-average inflammation of treated} \times 100 \quad (7)$$

$$\text{Average inflammation of control}$$

$$\%\ \text{inhibition of inflammation} = \frac{(\text{ VT –Vo})\text{control} - (\text{ VT} - \text{V0 }) \text{ treated groups}}{(\text{VT} - \text{V0})\text{control}} \times 100 \quad (8)$$

2.8.2. 2% Formaldehyde Induced Arthritis Model

This experiment was carried out using adult Wistar albino rats weighing 60–100 g. The animals were placed at random and allocated to treatment groups. They were housed in clean cages and allowed free access to food and water while they acclimatized to the laboratory environment one week prior to the experiment. A total of 32 animals were used and were divided into 8 groups of 4 rats each (n = 4). Group 1 received the vehicle water and served as the control group. Group 2 were induced with 0.1 mL of 2% formaldehyde but received no treatment. Group 3 received the standard diclofenac (20 mg/kg/body weight), group 4 received blank liposphere while groups 5, 6, 7 and 8 received doses of *Pentaclethra macrophylla* lipospheres (50, 100, 150, 200 mg/kg bodyweight), respectively, representing PM liposphere batches 2.5, 5, 7.5 and 10 g. One hour after the administration of oral doses of the vehicle and drugs, 0.1 mL of formaldehyde (2% v/v) was used to induce arthritis in the sub-plantar region of the right hind paw of all the rats [25].

After the intravenous 0.1 mL formaldehyde (2% v/v) administration, the volume of the paws was measured using a vernier caliper at 0, 1, 2, 3, 4, 5 and 48 h. Formation of oedema was then assessed in terms of the difference in the zero time paw edema volume of the injected paw and its paw edema volume at different times after 0.1 mL formaldehyde injection of the right hind paw. The % inhibition of the paw edema was calculated using the following formula:

$$\% \text{ inhibition of paw oedema} = 1 - \frac{\Delta V\text{treated}}{\Delta V\text{untreated}} \times 100 \quad (9)$$

where ΔV treated= mean change in paw volume edema of treated rat,

ΔV untreated = mean change in paw volume of untreated rat.

After 48 h, 2 mL of blood was collected by retro-orbital cavity under the influence of anesthesia. Aliquot of the collected blood was used to perform hematological studies to estimate total blood count and differential blood count, including eosinophils, basophils, lymphocytes, monocytes.

Total White Blood Cell Count

This was carried out following standard hematological procedures [26]. Briefly, well-mixed anticoagulated blood was diluted 1:20 with Truk's solution (2% glacial acetic acid) in a test tube. This was loaded into an improved Neubauer counting chamber. Appropriate squares were counted and added up to determine the total red cell count.

Differential Blood Count

Drops of blood (2 drops) were placed on a dry slide and spread on the surface using another slide (blood film method) [26]. The Leishman's stain was carefully dropped onto the blood film and was covered using a smaller slide. The stain was allowed to act on the blood for 2–3 min. After that, distilled water was used to wash off the stain until a pink color was obtained. Excess water was shaken off, underneath of the slide cleaned and the slide allowed to dry upright. The slide (smear side up) was placed on the microscope stage. The blood smear was examined under the microscope with ×10 lens resolution. The area with plenty WBCs was selected and an immersion oil was placed on that area. The resolution was changed to the oil immersion objective (100×). The differential cell count was then carried out and the morphology of the WBC noted.

3. Results and Discussion

3.1. pH Time Dependent Analysis

Time dependent pH analysis of lipospheres is presented in Table 2. There was no change in pH for the PM-unloaded lipospheres and 2.5 g of PM-loaded lipospheres from day 1 to day 30. However, in higher PM loadings, there were only slight changes in pH from day 1 to day 7. This change was highest in the 7.5 g PM lipospheres.

Table 2. Properties of the formulated lipospheres.

Liposphere Batches (g)	Particle Size (μm)	Encapsulation Efficiency (EE %)	Loading Capacity (LC)	pH Time Dependence (Days)		
				1	7	30
0	12.56 ± 2.18	-	-	3.5	3.5	3.5
2.5	26.87 ± 4.21	35.2	4.4	3.8	3.8	3.8
5.0	42.42 ± 3.64	75.6	1.89	3.8	3.9	3.9
7.5	82.27 ± 6.24	89.4	3.4	3.7	3.9	3.9
10.0	98.67 ± 10.23	94.1	4.7	3.7	3.8	3.8

3.2. Encapsulation Efficiency (EE) and Loading Capacity (LC)

Results of EE and LC are also presented in Table 2. EE ranged from 35% to 94%. It increased consistently and progressively from 35.2% for 2.5 g PM-loaded lipospheres to 94% in 10 g PM lipospheres. This trend, however, was not obtainable in the LC. An increase in PM loading from 2.5 g to 5.0 g led to a sharp fall in LC from 4.4 to 1.89; on further increase in PM loading to 7.5 g, LC quickly rose back to 3.4 and finally to 4.7 at 10 g PM loading.

3.3. Particle Size and Morphology of Lipospheres

The photomicrographs of the lipospheres in Figure 1 show that the particles were spherical in shape. The particle sizes were within the acceptable range for lipospheres. The particle size of the unloaded lipospheres was lower than those of the PM-loaded

liposphères. The result also shows that the particle size increased with an increase in the amount of PM loaded in the liposphères. This confirms that the PM is molecularly entrapped in the liposphères.

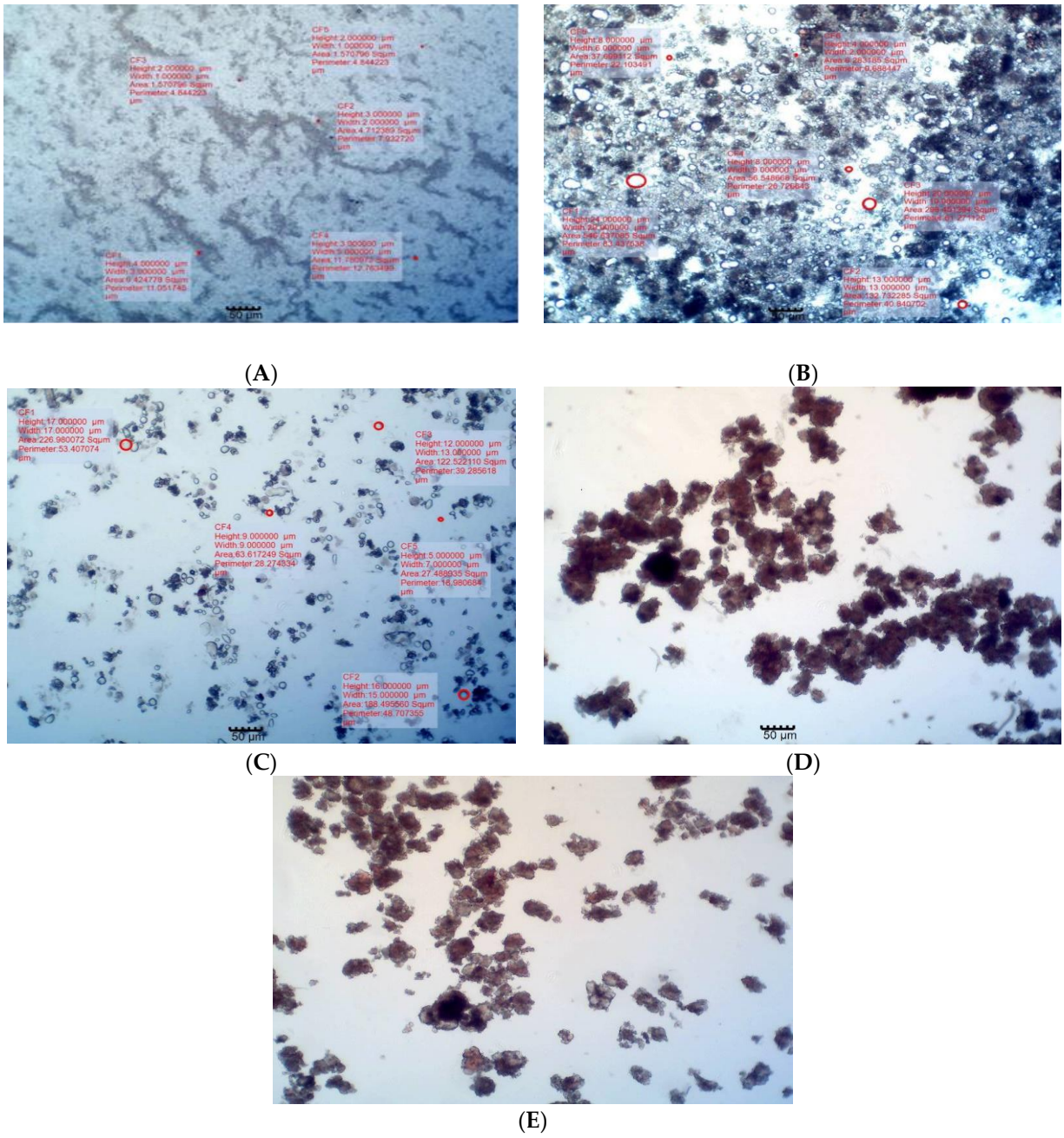
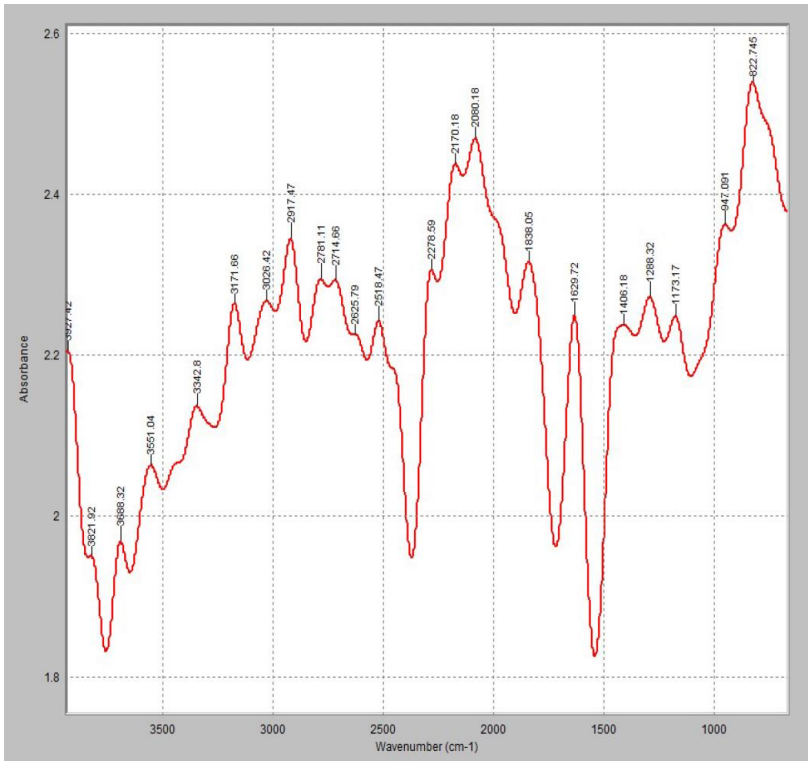


Figure 1. Morphology of the liposphères. (**A**) = Unloaded (placebo) liposphère; (**B**) = 2.5 g PM-loaded liposphère; (**C**) = 5.0 g PM-loaded liposphère; (**D**) = 7.5 g PM-loaded liposphère; (**E**) = 10.0 g PM-loaded liposphère.

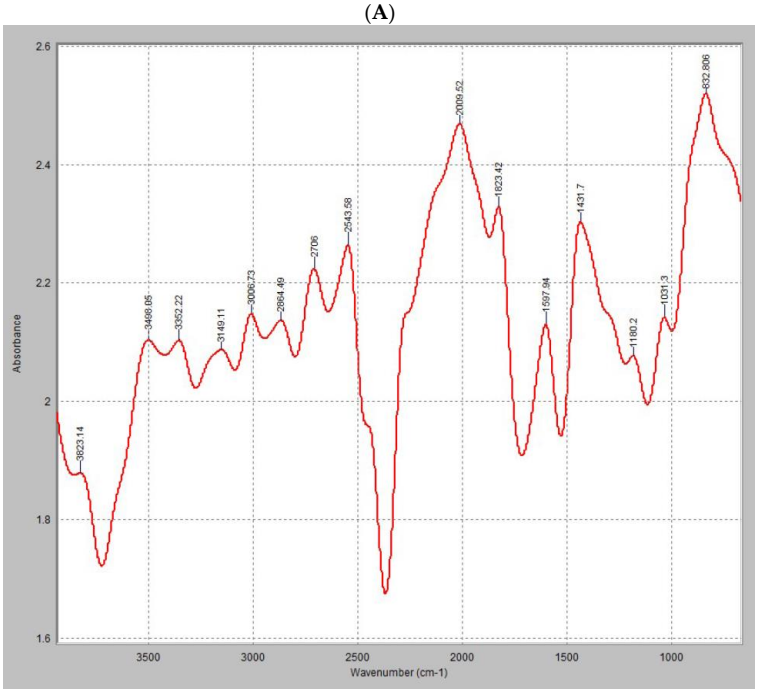
3.4. FTIR Analysis

An FTIR measures a sample's absorbance of infrared light at various wavelengths to determine the molecular composition and structure of the material. The results of the FTIR of the unloaded and loaded samples of the liposphères are shown in Figure 2. The unloaded sample maintained peaks at 3623.14 cm^{-1} (N-H groups), 27,706 cm^{-1} (C=O groups), 2009.92 cm^{-1} (C-O groups) and 832.9 cm^{-1} (C-H groups). In the fingerprint region of the extract, the PM-unloaded showed spectra with absorbance at 1457.4 cm^{-1} and 1379.1 cm^{-1}.

These peaks at this region corresponded to the asymmetric and symmetric bending of the C-H bond of methylene. An asymmetric and symmetric stretching of the C-H bond of methylene also occurred between 2935–2915 cm^{-1} and 2865–2845 cm^{-1} in all the formulated lipospheres samples. The loaded lipospheres had more sharp peaks at 2609 cm^{-1} (C=O groups) and 2002.75 cm^{-1} (C-O groups). Hence, the excipients used were compatible with the extract and there was no chemical interaction, thereby improving the stability of the formulations.

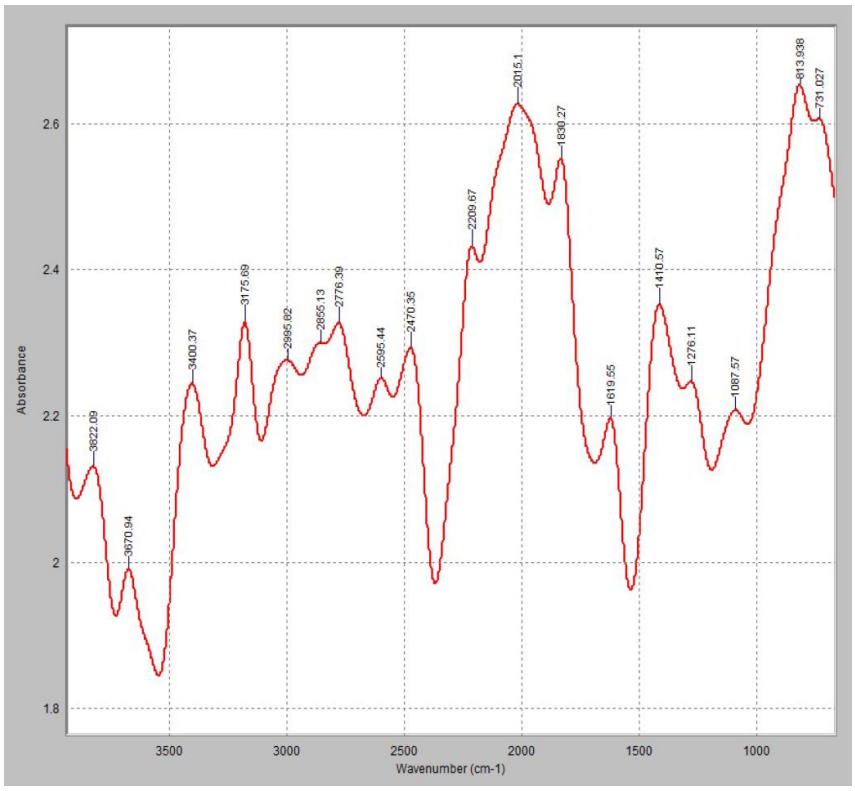


(**A**)

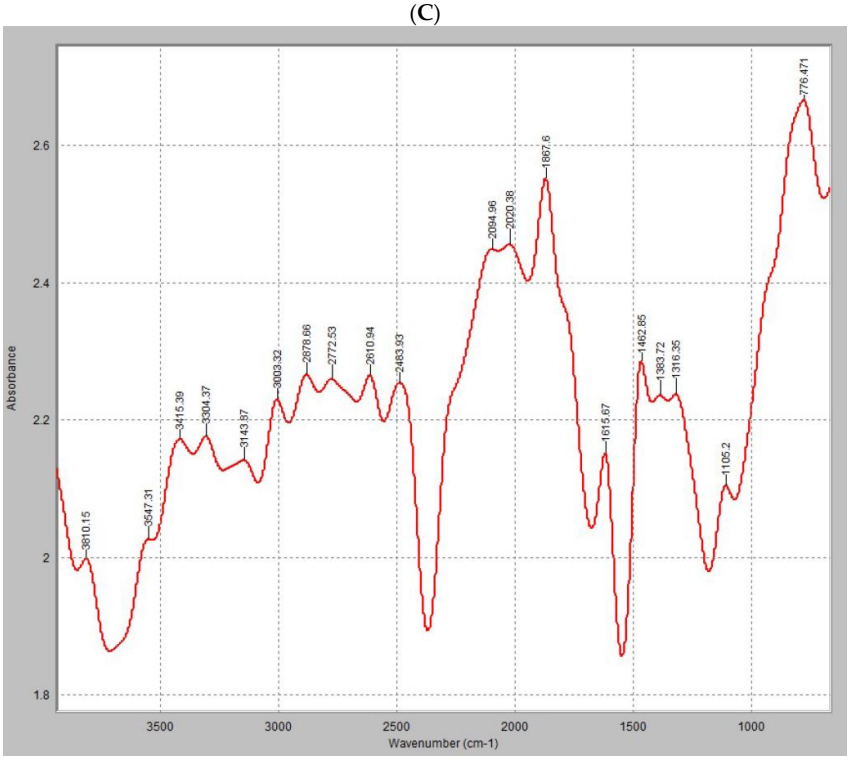


(**B**)

Figure 2. *Cont.*

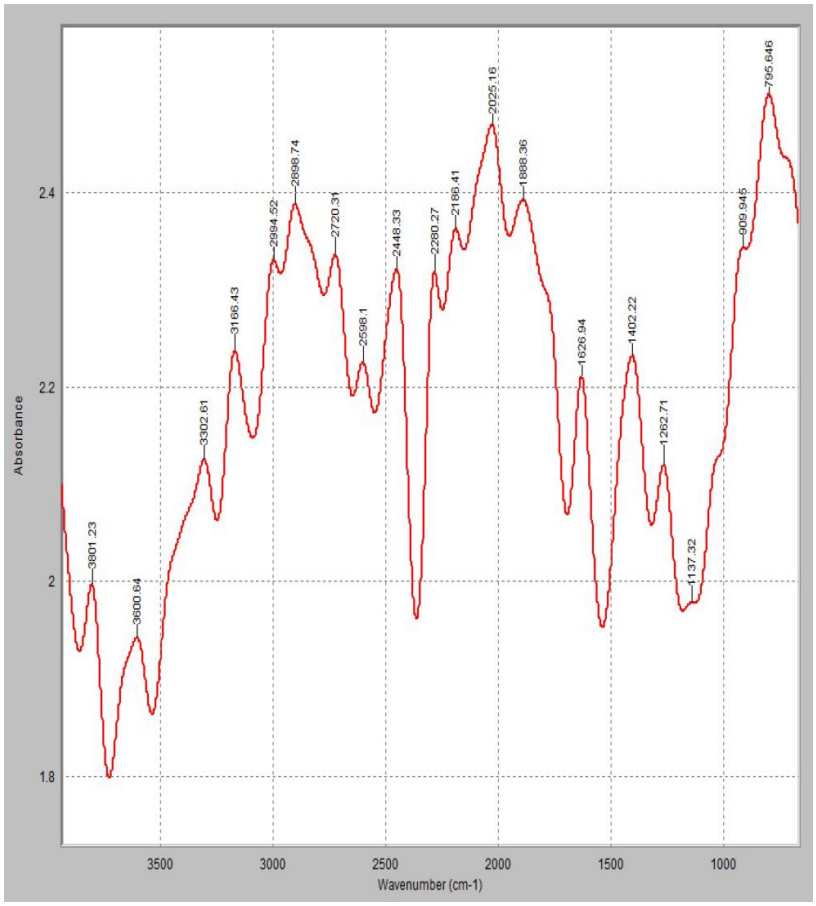


(**C**)



(**D**)

Figure 2. *Cont.*



(**E**)

Figure 2. FTIR spectra of lipospheres. (**A**–**E**) represent 0, 2.5, 5, 7.5, 10 g PM-loaded lipospheres.

3.5. In Vitro Anti-Inflammatory Studies

3.5.1. Membrane Stabilization

In Table 3, the percentage (%) stabilization of membrane by the samples and the standard drug is shown. The percentage protection of the membrane increased steadily with an increase in the concentration of PM in the lipospheres and the standard drug up to 400 µg/mL (300%). However, at 800 µg/mL, the percentage protection of the membrane reduced to 200%, which was higher than aspirin, the standard drug, at the same concentration. The higher the percentage inhibition, the more potent the concentration to stop the lysing of the red blood cells. The integrity of the cells is needed to maintain proper cellular function. In inflammation, the lysis of some cellular component leads to the release of inflammatory mediators. Stabilizing the red blood cell is a model to demonstrate the ability of the sample to stabilize those membranes of the stores of inflammatory mediators. Antioxidants play a good role in this. The sample contains antioxidant activity which could account for the anti-inflammatory activity [27]. When erythrocytes are exposed to hypotonic medium, heat, methyl salicylate, phenylhydrazine, among others, the lysis of their membrane occurs. It results in the leakage of serum protein and fluids into the tissues, instigating inflammation [21,27]. Membrane stabilization leads to the prevention of the leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators [27]. Therefore, the extracts are capable of membrane stabilization up to a dose of 400 µg/mL and are very suitable as anti-inflammatory agents [21,28]. The hypotonicity-induced hemolysis of erythrocytes is widely used as a simple, rapid, economic and sensitive tool in determining the anti-inflammatory property of drugs [21]. Because of its simplicity and economy, researchers prefer to use this model in the preliminary screening of medicinal plant extracts.

Table 3. Membrane stabilization of the samples and the standard drug.

Sample (µg/mL)	% Protection of Membrane
50	24.60
100	51.70
200	100.0
400	300.0
800	200.0
Aspirin (µg/mL)	
50	89.00
100	5.90
200	100.00
400	100.00
800	106.25

In other words, the crude methanol extract of *Pentaclethra macrophylla* which contains copious amounts of bergenin demonstrated noticeable membrane stabilizing properties [5]. Many flavonoids and triterpenes have been earlier reported to have anti-inflammatory properties [29]. Since flavonoids and triterpenes are also present in the *Pentaclethra macrophylla*methanol extract [30], it might be a reason for its membrane stabilizing anti-inflammatory potential.

3.5.2. Anti-Platelet Aggregation Activity

In Table 4, the percentage anti-platelet aggregatory activity of platelets by the samples and the standard drug is shown. Due to the endothelium's antiadhesive property, platelets move near to it during normal physiological conditions without creating a persistent adhesion contact. However, they can become unusually overactive in certain physiopathological conditions [31] and cause blood clots to form, which can cause thrombosis and increase the risk of cardiovascular and cerebrovascular problems [32]. Platelets are instantly exposed to subendothelial collagen, which is a significant thrombogenic component of this layer, when the endothelium is injured in the case of a vascular injury. Platelets then establish long-lasting adhesion connections with this collagen and other macromolecules used as adhesives [33]. In the present study, the platelet aggregation decreased steadily with an increase in the concentration of the PM in the lipospheres, as well as the standard, confirming the anti-inflammatory actions of the extract. However, 800 µg/mL of the sample drastically reduced aggregation without any effect on the platelet. Antiplatelets inhibit platelet aggregation, as observed in the inflammation.

The aim is to enable platelets to generate some inflammatory mediators that increase inflammation; the mechanism of anti-platelet aggregation could be due to indirectly inhibiting cyclooxygenase-2 or by the blockade of calcium influx through membrane calcium channels [31–33]. The model used showed that PM blocked calcium influx at the acute time, as shown by the result in Table 4.

Table 4. Anti-platelet aggregation activity.

Sample (µg/mL)	Change 1	Change 2	Change 3	Change 4
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
50	0.007 ± 0.0025^{cd}	0.0043 ± 0.0013^{bc}	0.0017 ± 0.0007^{bc}	0.001 ± 0.00^{b}
100	0.004 ± 0.001^{abcd}	0.0047 ± 0.0017^{bc}	0.005 ± 0.0021^{ab}	0.0037 ± 0.002^{a}
200	0.0013 ± 0.009^{ab}	0.002 ± 0.0006^{ab}	0.007 ± 0.0026^{a}	0.0023 ± 0.009^{b}
400	0.0017 ± 0.002^{ab}	0.0033 ± 0.0007^{bc}	0.0007 ± 0.0003^{c}	0.0057 ± 0.002^{a}
800	0.000 ± 0.00^{a}	0.000 ± 0.00^{a}	0.00 ± 0.00^{c}	0.001 ± 0.001^{b}

Table 4. *Cont.*

Sample (µg/mL)	Change 1	Change 2	Change 3	Change 4
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Aspirin (µg/mL)				
50	0.005 ± 0.001 ^{abcd}	0.0063 ± 0.0018 ^{c}	0.0023 ± 0.009 ^{bc}	0.002 ± 0.006 ^{b}
100	0.0033 ± 0.0007 ^{abc}	0.002 ± 0.0006 ^{ab}	0.0010 ± 0.00 ^{c}	0.0010 ± 0.006 ^{b}
200	0.004 ± 0.0021 ^{abcd}	0.003 ± 0.001 ^{abc}	0.003 ± 0.00 ^{bc}	0.0013 ± 0.0007 ^{b}
400	0.0057 ± 0.002 ^{bcd}	0.0043 ± 0.0015 ^{abc}	0.0027 ± 0.0007 ^{bc}	0.0013 ± 0.0003 ^{b}
800	0.009 ± 0.0012 ^{d}	0.0067 ± 0.0015 ^{c}	0.0027 ± 0.0007 ^{bc}	0.0023 ± 0.0009 ^{b}

SEM = standard error of mean (n = 5). In each column, values with same superscript do not differ significantly from each other at $p < 0.05$. Change 1 is absorbance at 30 s minus absorbance at zero sec; change 2 is absorbance at 60 s minus absorbance at 30 s; change 3 is absorbance at 90 s minus absorbance at 60 s; change 4 is absorbance at 120 s minus absorbance at 90 s.

3.6. In Vivo Anti-Inflammatory Studies

3.6.1. 2% Formaldehyde-Induced Arthritis Model

The effect of *Pentaclethra macrophylla* herbal lipospheres on an arthritis-induced model of inflammation is as shown in Table 5. The PM-loaded lipospheres inhibited edema consistently throughout the duration of observation. Among the extracts, 150 mg/kg (corresponding to the 7.5 g PM-loaded liposphere) had the highest inhibition of edema. There were significant differences between the test and positive controls. Formaldehyde-induced arthritis is a suitable model used in examining the anti-proliferative capability of anti-arthritic drugs. Formaldehyde induces arthritis by causing protein breakdowns at the injection site, which results in an immunological reaction [34]. There are two phases of formaldehyde's arthritic activity. Substance P is released during the early phase (0–5 min), while the late phase (15–30 min) sees the production of bradykinin, histamine, serotonin and prostaglandin, which causes a significant increase in permeability and vasodilation [34,35]. The nerve endings and pain receptors are stimulated by these mediators, which causes hyperalgesia. Thus, the injection site becomes hypersensitive [35,36]. Studies have shown that drugs with central nervous system activity consistently impair both phases, although agents with peripheral activity impair the late phase [36,37]. From the results obtained in the formalin test, the extract exhibited antinociceptive effects in both phases, thus confirming a peripheral mechanism of action while also suggesting the involvement of a central mechanism of analgesic action. Arthritis, which is marked by pains around the joints, could be as a result of auto-immunity, which could be genetic or by excessive deposition of metabolites, leading to gout. *Pentaclethra macrophylla* has been reported to have an anti-inflammatory potential. Farrukh et al. [38] have implicated down-regulation of the expression of genes for COX-2, PGE2, IL-1β, IL-6, TNF-α and NF-kβ and up-regulation of the expression of IL-4 and IL-10.

Table 5. The effect of *Pentaclethramacrophylla*-loaded lipospheres on arthritis model of inflammation in rats.

Treatments (mg/kg)	Edema (mL) ± SEM over Time (0–48 h)						
	0	1	2	3	4	5	48
T50	0.375 ± 0.025	0.750 ± 0.029 (0.00)	0.650 ± 0.029 ^{a} (7.14)	0.525 ± 0.025 ^{a} (0.00)	0.500 ± 0.00 ^{b} (16.7)	0.500 ± 0.00 ^{b} (0.00)	0.425 ± 0.048 (−6.2)
T100	0.300 ± 0.00	0.700 ± 0.00 (6.7)	0.600 ± 0.00 ^{b} (14.3)	0.525 ± 0.025 ^{a} (0.00)	0.525 ± 0.025 ^{b} (12.5)	0.475 ± 0.025 ^{c} (0.00)	0.400 ± 0.00 (0.00)
T150	0.325 ± 0.025	0.675 ± 0.025 (10.0)	0.575 ± 0.025 ^{b} (17.8)	0.525 ± 0.025 ^{a} (0.00)	0.525 ± 0.025 ^{b} (12.5)	0.500 ± 0.00 ^{b} (0.00)	0.425 ± 0.025 (−6.25)
T200	0.325 ± 0.025	0.725 ± 0.025 (3.33)	0.600 ± 0.00 ^{b} (14.29)	0.500 ± 0.00 ^{b} (4.8)	0.525 ± 0.025 ^{b} (12.5)	0.550± 0.029 ^{b} (10.0)	0.425 ± 0.025 (−6.25)

Table 5. *Cont.*

Treatments (mg/kg)	Edema (mL) ± SEM over Time (0–48 h)						
	0	**1**	**2**	**3**	**4**	**5**	**48**
PC	0.325 ± 0.025	0.70 ± 0.04 (6.7)	0.60 ± 0.04 ^{b} (6.7)	0.50 ± 0.041 ^{b} (4.77)	0.475 ± 0.00 ^{b} (21.0)	0.40 ± 0.00 ^{a} (20.0)	0.40 ± 0.00 (0.00)
NC	0.30 ± 0.00	0.75 ± 0.041	0.70 ± 0.029 ^{a}	0.525 ± 0.025 ^{a}	0.60 ± 0.00 ^{a}	0.50 ± 0.00 ^{b}	0.40 ± 0.00
Normal	0.35 ± 0.05	0.75 ± 0.05	0.65 ± 0.00 ^{a}	0.60 ± 0.00 ^{a}	0.5 ± 0.025 ^{b}	0.475 ± 0.025 ^{c}	0.40 ± 0.00

T50, T100, T150, T200 = treatments corresponding to the PM-loaded liposphere containing 2.5, 5, 7.5 and 10 g of PM; PC = positive control (Diclofenac 20 mg/kg); NC = negative control; Normal = no induction plus vehicle. SEM = standard error of mean (n = 5). In each column, values with same superscript do not differ significantly from each other at $p < 0.05$. NB: The values in parenthesis represent the percentage inhibition of arthritis.

3.6.2. Egg Albumin-Induced Rat Paw Edema Inflammatory Model

Table 6 shows the effect of *Pentaclethra macrophylla* herbal lipospheres on egg albumin-induced inflammation in rats. Histamine and serotonin are released at the early phase as a result of egg albumin, which causes edema [39]. The test substances at 50 and 100 mg/kg did not inhibit the inflammation throughout the observation time. This is clearly shown by a negative percentage inhibition across all the time intervals. At a dose of 150 mg/kg, there was slight inhibition initially; after 2 h, the inhibition disappeared, as shown by the negative percentage inhibition at 3 h. This same trend was seen in the negative control, which shows no justifiable and significant differences in activity when compared to the test samples at the different doses. Pentaclathra has been reported to have anti-inflammatory properties [39], and Farrukh et al. [38] has implicated down-regulation of the expression of genes for COX-2, PGE2, IL-1β, IL-6, TNF-α and NF-kβ and up-regulation of the expression of IL-4 and IL-10.

Table 6. Effect of *Pentaclethra macrophylla*-loaded lipospheres on Egg albumin-induced inflammation in rats.

Treatments (mg/kg)	Edema (mL) ± SEM over Time (h)					
	0	**1**	**2**	**3**	**4**	**5**
T50	0.350 ± 0.029	0.625 ± 0.025 (−4.17)	0.550 ± 0.029 ^{abc} (−4.7)	0.500 ± 0.058 ^{a} (−17.65)	0.475 ± 0.048 ^{a} (−15.79)	0.650 ± 0.029 ^{a} (−8.33)
T100	0.275 ± 0.025	0.625 ± 0.025 (−4.17)	0.600 ± 0.00 ^{ab} (−14.29)	0.525 ± 0.025 ^{a} (−23.5)	0.525 ± 0.025 ^{b} (−31.25)	0.650 ± 0.029 ^{a} (−4.17)
T150	0.300 ± 0.00	0.500 ± 0.00 (16.6)	0.500 ± 0.058 ^{c} (4.76)	0.433 ± 0.033 ^{b} (−1.88)	0.433 ± 0.33 ^{a} (−8.25)	0.633 ± 0.033 ^{a} (−5.5)
T200	0.267 ± 0.033	0.600 ± 0.058 (0.00)	0.533 ± 0.033 ^{ac} (−1.5)	0.500 ± 0.00 ^{a} (−17.64)	0.533 ± 0.033 ^{b} (−33.25)	0.700 ± 0.00 ^{a} (−6.0)
PC	0.30 ± 0.00	0.625 ± 0.025 (4.17)	0.500 ± 0.00 ^{c} (4.76)	0.425 ± 0.025 ^{b} (0.00)	0.425 ± 0.025 ^{a} (−6.25)	0.600 ± 0.00 ^{b} (0.00)
NC	0.30 ± 0.00	0.6 ± 0.00	0.525 ± 0.025 ^{b}	0.425 ± 0.029 ^{a}	0.400 ± 0.00 ^{a}	0.60 ± 0.00 ^{a}
Normal	0.275 ± 0.025	0.55 ± 0.029	0.625 ± 0.025 ^{ac}	0.575 ± 0.025 ^{b}	0.50 ± 0.041 ^{a}	0.65 ± 0.029 ^{b}

T50, T100, T150, T200 = Treatments corresponding to the PM-loaded liposphere containing 2.5, 5, 7.5 and 10 g of PM; PC = positive control (Diclofenac 20 mg/kg); NC = negative control; Normal = no induction plus vehicle. SEM = standard error of mean (n = 5). In each column, values with same superscript do not differ significantly from each other at $p < 0.05$. NB: The values in parenthesis represent percentage inhibition of inflammation.

3.6.3. Blood Cell Counts of Infected Rats

The effect of *Pentaclethra macrophylla*-loaded lipospheres on the blood cell profiles of infected rats is as shown in Table 7. The T50 dose of PM had the highest percentage of WBC, and it decreased as the treatment doses increased from T100 to T200. There were no significant differences among the Neutrophil counts of the different groups. Similar trends were observed in the lymphocytes and Eosinophil counts. The monocytes and the basophils were undetected.

Table 7. Effect of *Pentaclethra macrophylla* herbal lipospheres on the blood cell counts of infected rats.

Treatments (mg/kg)	Percentage Compositions of Different Blood Cells Count ± SEM					
	WBC (%)	Neutrophil (%)	Lymphocytes (%)	Eosinophil (%)	Monocyte (%)	Basophil (%)
T50	77.50 ± 3.59	65.50 ± 1.50 ^{bc}	34.00 ± 1.414 ^{abc}	0.50 ± 0.5	0.00	0.00
T100	70.00 ± 1.83	58.50 ± 1.71 ^{a}	40.00 ± 1.63 ^{c}	1.50 ± 0.5	0.00	0.00
T150	70.50 ± 1.71	61.50 ± 1.71 ^{abc}	37.50 ± 2.22 ^{abc}	1.00 ± 0.57	0.00	0.00
T200	67.50 ± 3.50	61.00 ± 1.29 ^{b}	38.50 ± 0.96 ^{bc}	0.50 ± 0.5	0.00	0.00
PC	71.00 ± 3.512	63.50 ± 3.403 ^{abc}	36.00 ± 3.747 ^{abc}	0.50 ± 0.50	0.00	0.00
NC	67.00 ± 2.646	67.25 ± 0.479 ^{c}	31.00 ± 0.577 ^{a}	1.75 ± 0.629	0.00	0.00
Normal 1	72.00 ± 4.320	61.50 ± 1.50 ^{abc}	36.50 ± 0.751 ^{abc}	2.0 ± 0.816	0.00	0.00
Normal 2	68.80 ± 2.062	65.00 ± 1.915 ^{bc}	32.00 ± 1.414 ^{ab}	2.50 ± 0.500	0.00	0.00

T50, T100, T150, T200 = Treatments corresponding to the PM-loaded liposphere containing 2.5, 5, 7.5 and 10 g of PM; PC = positive control (Diclofenac 20 mg/kg); NC = negative control; Normal 1 = no induction, no treatment; Normal 2 = no induction plus vehicle. SEM = standard error of mean (n = 5). In each column, values with same superscript do not differ significantly from each other at $p < 0.05$.

4. Conclusions

There are associated side effects with the delivery systems and anti-inflammatory agents currently in use. There is, therefore, a need to investigate the potency of herbal lipospheres from *Pentaclethra macrophylla*. The excipients used were compatible with the extract and there was no chemical interaction, thereby improving the stability of the formulations. The characterization of the herbal lipospheres presented good qualities and, hence, a promising delivery system for the herbal formulation of *Pentaclethra macrophylla*, which is a potent drug that ameliorates different ailments. The herbal lipospheres produced a significant inhibition of hemolysis in vitro by membrane stabilization, while platelet aggregatory inhibition decreased with an increase in the concentration of extract in the lipospheres. The PM-loaded liposphere inhibited edema consistently during the period of observation. The study therefore showed that *Pentaclethra macrophylla* was able to produce efficient herbal lipospheres with antinociceptive and anti-inflammatory activities.

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