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Lipospheres: A Potential Delivery System of Herbal Extract for the Treatment of Diabetes Mellitus

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ABSTRACT

In view of the wide spread use of herbal formulations and its proven efficacy, there is need for standardization and quality control. *Gongronema latifolium* is widely used in the folk medicine in the treatment of diabetes, hence the need for an effective delivery system for this natural plant. The methanolic extract of *Gongronema latifolium* leaves (1, 3 and 5 %) were formulated into lipospheres by melt homogenization using lipid matrix consisting of mixture of goat fat and Phospholipon® 90H (1:2). The lipospheres were characterized by analyzing the encapsulation efficiency(EE), pH, particle size and morphology. The *in vivo* antidiabetic properties were studied in alloxan induced diabetic Wistar rats. The results showed spherical lipospheres within µm limit, stable pH over 30 days and EE% of 86, 89 and 90 % for batches A, B and C containing 1, 3 and 5 % *Gongronema latifolium* extract. *Gongronema latifolium*-loaded lipospheres (100 mg/kg) had 55.2 and 70.6 % reduction in blood glucose of the diabetic rats at 8 and 12 h respectively, while, the rats that received glibenclamide (0.2 mg/kg) had 38.4 and 53.4 % reduction in blood glucose at 8 and 12 h. *Gongronema latifolium*-loaded lipospheres had significantly higher antidiabetic properties than ($p < 0.05$) the reference drug at concentrations used.

Keywords: Alloxan induced diabetes, *Capra hircus* fats, *Gongronema latifolium*, lipospheres

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INTRODUCTION

In view of the popular and widespread use of herbal plants in the management of diabetes, important technical aspects such as standardization and quality control will be of immense benefit in order to enhance their efficacy and improve patient's compliance [1-2.]

Gongronema latifolium Benth Hook, (Asclepiadaceae) is a herbaceous shrub whose leaf extract is used in the treatment of malaria, diabetes, hypertension, and as laxative [3]. It is also used as a spice and vegetable [4]. The use of crude leaf extract of this shrub in maintaining healthy blood glucose levels has been reported [3, 5]. *Gongronema latifolium* commonly called 'utazi' and 'arokeke' in the South Western and South Eastern parts of Nigeria, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [5-7]. The leaf extract is rich in proteins (27.2 %). Phytochemical analysis of leaf extract of *Gongronema latifolium* reveals the presence of essential oil, saponins, alkaloids, minerals with calcium, phosphorus, magnesium, copper and potassium [4-5, 8-10].

The rapid growth in the use of lipid-based drug delivery systems is primarily due to the diversity and versatility of pharmaceutical grade lipid excipients and their compatibility with liquid, semi-solid and solid dosage forms [11]. The proven safety (biocompatibility) of lipid based carriers makes them attractive candidates for the formulation of pharmaceuticals. The widening availability of lipidic excipients with specific characteristics offer flexibility of application with respect to improving the bioavailability of poorly water soluble drugs and manipulating their release profile [12]. Lipid-based formulations can be used to influence the absorption of active ingredients through different mechanisms to modify the release of active ingredients thus, improving bioavailability. They can affect the intestinal environment, stimulate the lymphatic transport of active ingredients, and interact with enterocyte based transport [13].

Lipospheres were first reported as a particulate dispersion of solid spherical particles between 0.2-100 μm in diameter consisting of solid hydrophobic fat core such as triglycerides or fatty acids derivatives, stabilized by monolayer of phospholipids [14]. The internal core contains the drug dissolved or dispersed in solid fat matrix. Liposphere drug delivery system is an emerging carrier for both hydrophilic and hydrophobic drugs and has several advantages over other delivery systems in terms of physical stability, low cost of ingredients, ease of preparation, and scale-up, high dispersibility in aqueous medium, high entrapment efficiency, and extended release of entrapped drug [14-15]. Most biotech drugs and plant-derived bioactive compounds can be formulated as lipospheres [16-17]. Liposphere formulation could be a better way to formulate

plant extracts for increased bioavailability [18]. The aim of the present study is to investigate the properties of *Gongronema latifolium* extract in lipospheres and to study *in vivo* the antidiabetic properties of the formulations in alloxan induced diabetic rats.

MATERIALS AND METHODS

Materials

Solutol® (BASF, Germany), Phospholipon® 90H (Phospholipid GmbH, köln, Germany), activated charcoal (Bio-Lab (UK) limited, London), distilled water (STC UNN, Nigeria), methanol, sorbic acid, sorbitol (Merck, Darmstadt, Germany). Goat fat was extracted from *Capra hircus* and *Gongronema latifolium* leaves were collected from a thick bush at Edem-ani in Nsukka, Enugu state, Nigeria in the month of March, 2012 and were authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethno medicine and Drug Development (InterCEDD) Nsukka. The leave were extracted and processed in our laboratory.

Extraction of fat from *Capra hircus*

The fat was extracted by grating the adipose tissue prior to boiling 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. Further purification was carried out by heating a 2% w/w suspension of a 1:9 ratio blend of activated charcoal and bentonite in the lipid at 80 to 90°C for 1 h. Thereafter, the suspension was vacuum-filtered using Buchner funnel [19].

Extraction of *Gongronema latifolium*

Gongronema latifolium leaves were dried under the shade For 5 days and the dried leaves were milled using a blender (500# grinder/Fuyu Metal, Linyi Fuyu Metal Products Co., Ltd, China). The powdered *G. latifolium* leaves were extracted by cold maceration using methanol as extracting solvent. The leaves were soaked in 1200 ml of analytical methanol for 72 h with constant shaking for the first 6 h. The cold macerate was filtered using a muslin cloth embedded with cotton wool and attached to a filter funnel. The residue was discarded and the filtrate was transferred into a stainless steel tray for the methanol to evaporate.

Preparation of lipid matrix

The lipid matrix was prepared by fusion using Phospholipon® 90H and purified goat fat at a ratio of 1:2.5. The lipids were weighed and melted together in a beaker placed on a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) at 70 ^{o}C and stirred with a glass stirrer until a transparent homogenous white melt was obtained. The lipid matrix was stirred continuously until it solidified at room temperature [19].

Preparation of lipospheres

The lipospheres were prepared by melt homogenization using an Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany). Details of the composition of the lipospheres are shown in Table 1. In each case 5 g of the lipid matrix was weighed using analytical balance (Adventurer, Ohaus, China), melted in a beaker at a temperature of 70 ^{o}C using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) and the appropriate amount of *G. latifolium* was incorporated into the lipid melt. Sorbitol was dissolved in hot distilled water at the same temperature with the lipidic melt together with Solutol® and sorbic acid. The hot aqueous phase was poured into the lipidic melt and immediately subjected to high shear homogenization with Ultra-Turrax at 5000 rpm for 10 min. Lipospheres containing no drug (unloaded lipospheres), which served as negative control was also formulated [20-21].

Determination of particle size and morphology

Small amount of lipospheres were placed on a microscope slide, the slide was covered with a cover slip and imaged under a Hund® binocular microscope (Weltzlar, Germany), attached with a motic image analyzer (Moticam, China) at a magnification of x100. Different particles of the lipospheres from each batch were measured (n=100), and the mean value was determined.

The pH studies

The pH of the lipospheres was determined in time dependent manner (24 hours, 1week, and 1month) using pH meter (Suntex TS-2, Taiwan).

Encapsulation efficiency and loading capacity

Beer's calibration curve was obtained for *G. latifolium* in simulated intestinal fluid (SIF, pH 7.2) at a concentration range of 0.1 – 1.0 mg% at a predetermined wavelength of 300 nm. Approximately 10 ml of the *G. latifolium*-loaded lipospheres was added into a centrifuge and into a centrifuge (Chem. Lab. Instrument, UK) and separated at 1,252 × g for 30 min. The supernatant were adequately analyzed in spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA). Drug content was calculated with reference to Beer's calibration curve.

The encapsulation efficiency (EE %) of the *G. latifolium* in the lipospheres was calculated from the equation below:

$$\text{EE}\ (\%) = \frac{\text{Wi} - \text{Wf}}{\text{Wi}} \text{x}\ 100 \qquad (1)$$

Where Wi is the mass of *G. latifolium* added to the formulation, while Wf is the actual mass of *G. latifolium* encapsulated in the lipospheres.

The LC was determined using the relationship

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

Where Wl is the weight of lipid in the formulation, Wa is the weight of *G. latifolium* added to the formulation and Ws is the actual amount of *G. latifolium* encapsulated in the lipospheres.

IN-VIVO ANTIDIABETIC STUDY

Animal protocol

Wistar albino rats of either sex weighing 180 - 200 g were procured from the Biochemistry Department, University of Nigeria, Nsukka and were maintained at standard housing conditions with 12 h light. *Ab initio*, the animals were fed with a commercial diet (Feeds BC, Nsukka, Nigeria) and water during the experiment. All animal experimental protocol was in accordance with the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka and in accordance with the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Induction of diabetes mellitus

The rats were fasted overnight prior to the induction of diabetes mellitus. Many experimental models have been used for studying diabetes mellitus [22-23]. Chemical induction with alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5, 6-pyrimidinetetrone), an oxygenated pyrimidine derivative [23], appears to be the easiest, most reliable practical method of inducing diabetes mellitus in rodents [24]. Blood was collected for baseline glucose determination [24]. Fresh solution of alloxan monohydrate (Sigma, USA) was prepared just prior to injection. A stock solution of alloxan monohydrate was made by dissolving alloxan in normal saline (0.9 w/v% NaCl) at a concentration of 100 mg/kg [24]. A volume equivalent to 1 ml of the stock solution was given intra-peritoneally after which the blood glucose levels were measured at regular intervals (i.e. every six hours) four times daily for three days using a glucometer (ACCU-CHECK, Roche, USA). Food consumption was measured in (g), water (ml), and urine volume (ml) on a daily basis. Diabetes was confirmed 3 days post-alloxan administration [24].

Oral administration of *G. latifolium*-loaded lipospheres

Four groups of animals of six rats per group were used for the test. The control group received normal saline (2 ml), the reference group received commercial sample of glibenclamide (Daonil®) (5 mg), while the test groups received *G. latifolium*-loaded lipospheres equivalent to 100 mg/kg *G. latifolium* reconstituted with water (0.5 ml) and given orally using a 1 ml syringe.

The fourth group received *G. latifolium* extract (100 mg/kg). The blood glucose was checked at predetermined time intervals of 0, 0.5, 1, 2, 4, 8, and 12 h.

Data and statistical analysis

Statistical analysis were performed using SPSS Version 16.0 (SPSS Inc. Chicago, IL.USA). Data were analyzed by one-way ANOVA. Differences between means were assessed using student's t-test.

RESULTS AND DISCUSSION

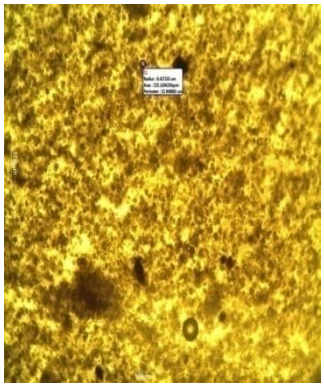
Particle size and morphology

The photomicrographs of the lipospheres are shown in Figure 1 and show that the particles were spherical in shape. The results of particle size of *G. latifolium*-loaded lipospheres are shown in Table 2. The particle sizes were within the acceptable range for lipospheres. The particle size of unloaded lipospheres was lower than that of the loaded lipospheres. The results also show that particle size increased with increase in the amount of *G. latifolium* loaded in the lipospheres. These results were in agreement with previous works done by researchers [25-26]. However, particle size of lipospheres may be affected by the formulation excipients degree of homogenization, homogenization pressure, rate of particle size growth, crystalline habit of the particle, etc. [11, 20].

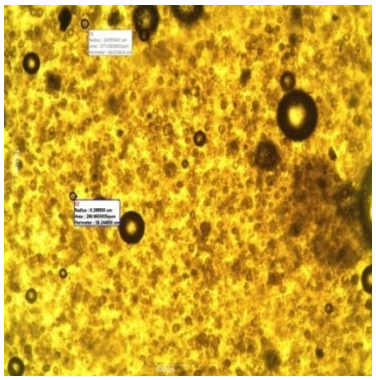
Table 1: : Composition of the *Gongronema latifolium*-loaded lipospheres

Batches Ingredient	A	B	C	D
Drug (*G. latifolium*) (%)	1	3	5	0
Lipid matrix (%)	5	5	5	5
Sorbitol (%)	4	4	4	4
Solutol® (%)	0.75	0.75	0.75	0.75
Sorbic acid (%)	0.05	0.05	0.05	0.05
Distilled water q.s. (%)	100	100	100	100

Batches A, B and C contain 1, 3 and 5 % of *Gongronema latifolium*, batch D: bland or unloaded lipospheres.



(a)



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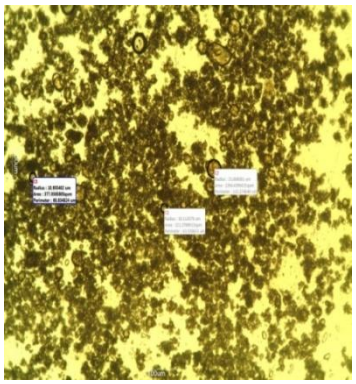

(c)

Figure. 1: The Photomicrographs of lipospheres: (a): unloaded lipospheres, (b): lipospheres loaded with 1 % *Gongronema latifolium*, (c): lipospheres loaded with 5 % *Gongronema latifolium.*

The pH of lipospheres

The pH of the lipospheres are shown in Table 2 and the results show that the unloaded or bland lipospheres (batch D) had a pH of 5.6 to 5, while that of the *G. latifolium* loaded lipospheres had pH of 6.5 to 5.6 at day 1 and 30 days respectively for batch A, containing 1 % *G. latifolium.* The results showed a general stable pH from 1 to 30 days in agreement with the work done by Brown et al, who studied the *in vitro* and *in vivo* characterization of piroxicam-loaded dika wax lipospheres [27]. There was a slight decrease in the pH of both the bland and *G. latifolium* loaded lipospheres. This decrease in pH with time was not due to degradation of *G. latifolium* in the lipospheres since there was also a decrease in the pH of the bland lipospheres. The pH decrease may be due to the release of free fatty acid from the lipid matrix [11].

Table 2: Physicochemical properties of *Gongronema latifolium*-loaded lipospheres

Batches	TDC (g)	ADC (g)	EE (%)	LC (%)	Particle size (µm ± SD)	pH 1 day	pH 7 days	pH 30 days
A	1	0.86 ± 1.12	86.0	2.72	20.225 ± 1.685	6.50 ± 0.05	6.00 ± 0.03	5.60 ± 0.11
B	3	2.67 ± 0.09	89.0	6.20	22.754 ± 2.528	6.10 ± 0.07	5.80 ± 0.05	5.20 ± 0.07
C	5	4.50 ± 1.17	90.0	9.10	21.068 ± 0.842	5.90 ± 0.11	5.60 ± 0.04	4.90 ± 0.09
D	0	-	-	-	16.860 ± 5.920	5.60 ± 0.11	5.30 ± 0.07	5.00 ± 0.05

Batches A, B and C contain 1, 3 and 5 % of *Gongronema latifolium*, batch D: bland or unloaded lipospheres, EE: Encapsulation efficiency; LC: Loading capacity.

The results of EE% and LC of *G. latifolium*-loaded lipospheres are shown in Table 2. From the results, EE% of 86, 89 and 90 % was obtained respectively for batches A, B and C containing 1, 3 and 5 % of *G. latifolium*. The results show that EE increased with increase in drug loading. The results of LC also showed that it increased with increase drug loading as shown in Table 2. The ability of the lipospheres to accommodate active molecules is an important property and this can be expressed by the EE% and LC. EE% defines the ratio between the weight of entrapped drug and the total weight of drug added to the dispersion, while LC expresses the ratio between the entrapped drug and the total weight of the lipids [11]. Both EE% and LC are dependent on several parameters, such as the lipophilic properties of the drug, the screening of the most appropriate lipid composition/ratio and surfactant combination, as well as the production procedures [11].

Hypoglycemic properties of *G. latifolium* lipospheres

The results of fasting blood glucose (FBG) level of the alloxan-induced diabetic rats are shown in Table 3. The results show that the reference drug 0.2 mg/kg glibenclamide had 175.50 ±

28.12, 116.50 ± 30.48, 108.2 ± 28.27 and 82.00 ± 38.34 mg/dl at 0, 4, 8 and 12 h respectively, the extract of *G. latifolium* had FBG of 208.50 ± 80.91, 135.75 ± 83.58, 117.25 ± 81.86 and 97.50 ± 8.51 mg/dl at 0, 4, 8 and 12 h, while *G. latifolium* lipospheres had FBG of 193.75±29.57, 121.25±48.71, 86.75±25.04 and 57.00±5.94 mg/dl at 0, 4, 8 and 12 h. The results showed that at 8 and 12 h, *Gongronema latifolium*-loaded lipospheres had significantly lower FBG than the reference drug ($p < 0.05$) and the pure extract ($p < 0.01$) at concentrations used. The results of the percentage reduction in blood glucose shown in Figure. 2 also showed that the *Gongronema latifolium*-loaded lipospheres had 55.2 and 70.6 % reduction in blood glucose of the diabetic rats at 8 and 12 h respectively, the group that received the extract of *Gongronema latifolium* had percentage blood glucose reduction of 44.0 and 53.0 % respectively while, the rats that received glibenclamide had 38.4 and 53.4 % reduction in blood glucose of the alloxan induced diabetic Wistar rats at 8 and 12 h. Therefore, *Gongronema latifolium*-loaded lipospheres had higher antidiabetic properties than the reference drug and the extract at concentrations used. This may be due to increased absorption of *Gongronema latifolium* in the presence of lipid. Also, the presence of surfactants may increase the absorption.

Table 3: Fasting blood glucose concentration (mg/dl)

Groups	0 h	0.5h	1 h	2 h	4 h	8 h	12 h
0.2 mg/kg Glibenclamide	175.50± 28.12	161.25± 14.97*	155.75± 15.65	145.00±1 8.99	116.50± 30.48	108.2±2 8.27	82.00±3 8.34
2 ml/kg Normal saline	331.25± 136.40	327.25± 131.93*	305.50± 126.21	254.00±1 12.93**	237.50± 109.67**	216.75± 102.33	205.50± 103.91
100 mg/kg pure extract	208.50± 80.91	196.25± 71.16	176.75± 84.15	158.25±9 6.89**	135.75± 83.58**	117.25± 81.86**	97.50±8 .51**
100 mg/kg drug formulation (Batch C)	193.75± 29.57	174.00± 46.20	160.50± 51.31	138.75±5 5.92	121.25± 48.71	86.75±2 5.04**	57.00±5 .94**

Values are expressed as the mean ± SEM, n = 6 **P < 0.05: significantly different compared to the reference drug glibenclamide 0.2 mg/kg, *P < 0.01: Significantly different compared to the pure extract.

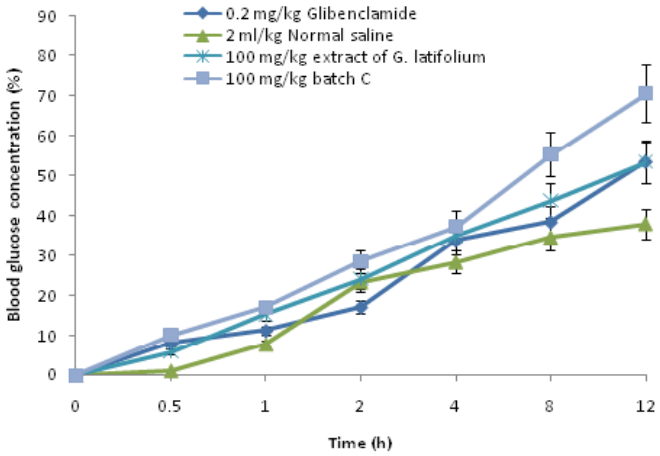

Figure 2: Results of percentage reduction in blood glucose with time in alloxan induced diabetic Wistar rats treated with *Gongronema latifolium*-loaded lipospheres (100 mg/kg), *Gongronema latifolium* extract (100 mg/kg), 2 ml/kg normal saline, and glibenclamide (0.2 mg/kg). *P < 0.05 was considered to be statistically significant compared to the reference drug glibenclamide; **P < 0.01 was considered to be statistically significant compared to the pure extract.

CONCLUSION

Gongronema latifolium-loaded lipospheres exhibited good particle size, stable pH and high encapsulation efficiency. The results revealed that the lipospheres loaded with *Gongronema latifolium* exhibited significantly higher reduction in blood glucose than the *Gongronema latifolium* extract and the reference drug-glibenclamide ($p < 0.05$) at concentrations used in the study. Therefore, *Gongronema latifolium* could be formulated as lipospheres in order to enhance the *in vivo* bioavailability of this natural drug so as to effectively manage diabetes.

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REFERENCES

1. Bonati A. How and why should we standardize phytopharmaceutical drugs for clinical validation. J Ethnopharm 1991; 32: 195-198.
2. Chime SA, Brown SA, Ugwu CE, Agubata CO, Obidike TC, Onunkwo GC. Effect of binder type and concentration on the *in vitro* properties of *Alstonia boonei* tablets. Int J Pharm Sci Rev Res 2012; 16(2): 5-9.

3. Edet EE, Akpanabiatu MI, Eno AE, Umoh IB, Itam EH. Effect of *Gongronema latifolium* crude leaf extract on some cardiac enzymes of alloxan-induced diabetic rats. Afr J Biochem Res 2009; 3(11): 366-369.
4. Morebise FMA, Makinde JM, Olajide OA, Awe EO. Anti-inflammatory property of the leaves of *Gongronema latifolium*. Phyto Res Supp 2002; 1: 75-77.
5. Udosen EO, Egbung GE, Onofiok UL, Robert AE, Ekam VS, Iwara AI, Odey MO. Effect of ethanolic root and twig extracts of *gongronema latifolium* (utazi) on kidney function of streptozotocin induced hyperglycemic and normal Wistar rats. J Med Med Sci 2012; 3(5): 291-296.
6. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoter 2002; 73: 612-618.
7. Ugochukwu NH, Babady NE, Coboume MK, Gasset SR. The effect of *Gongronema latifolium* extract on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J Biosci 2003; 28(1): 1-5.
8. Schneider C, Rotscheidt K, Breitmaier E. Four new pregnane glycosides from *Gongronema latifolium* (Asclepiadaceae). J für Praktische *Chem 1993;* 335(6): 532 – 536.
9. Eleyinmi AF, Bressler DC. Chemical composition, anti-bacterial and some hematological indices activity of *Gongronema latifolium*. J Zhej Uni Sci 2007; 8(5): 302-308.
10. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium*. Afr J Biotech. 2009; 8(18): 4685-4689.
11. Attama AA, Okafor CE, Builders PF, Okorie O. Formulation and in vitro evaluation of a PEGylated microscopic lipospheres delivery system for ceftriaxone sodium. J Drug Deliv 2009; 16: 448–616.
12. Attama AA, Nkemnele MO. In vitro evaluation of drug release from self micro-emulsifying drug delivery systems using a biodegradable homolipid from *Capra hircus*. Int J Pharm 2005; 304: 4–10.
13. Fouad EA, El-badry M, Mahrous GM, Alsarra IA, Alashbban Z and Alanazi FK. *In vitro* investigation for embedding dextromethorphan in lipids using spray drying. Digest J Nanomat Bio 2011; 6(3):1129-1139.

14. Domb AJ, Maniar M, Manoj. Lipospheres for controlled delivery of substances. European Patent 1996; EP0502119.
15. Singh Rawat M, Singh D, Swarnlata S. Development and in vitro evaluation of polar lipid based lipospheres for oral delivery of peptide drugs. Int J Drug Deliv 2009; 1: 15 – 26.
16. Barbosa CMS, Morais HA, Deivivo FM, Mansur HS, Oliveira MCD, Silvestre MPC. Papain hydrolysates of casein: Molecular weight profile and encapsulation in lipospheres. J Sci Food Agric 2004; 84: 1891 – 1900.
17. Sharma S, Roy RK. Phytosomes: An emerging technology. Int J Pharm Res Dev 2010; 2 (5): 1 – 7.
18. Chime SA, Kenechukwu FC, Onunkwo GC, Attama AA, Ogbonna JD. Recent advances in lipospheres drug delivery system. J Pharm Res 2012; 5(3): 1743-1748.
19. Obitte NC, Chime SA, Magaret AA, Attama AA, Onyishi IV, Brown SA. Some *in vitro* and pharmacodynamic evaluation of indomethacin solid lipid microparticles. Afr J Pharm Pharm 2012; 6(30): 2309-2317.
20. Chime SA, Attama AA, Builders PF, Onunkwo GC. Sustained release diclofenac potassium-loaded solid lipid microparticle based on solidified reverse micellar solution: *In vitro* and *in vivo* evaluation. J Micro 2012: 1–11. DOI: 10.3109/02652048.2012.726284.
21. Friedrich I, Müller-Goymann CC. Characterization of solidified reverse micellar solutions (SRMS) and production development of SRMS-based nanosuspension. Eur J Pharm Biopharm 2003; 56: 111-119.
22. Vivek KS, Suresh K, Hitesh, JP, Shivakumar H. Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. Int J Pharm Sci Rev Res 2010; 1(2): 18-22.
23. Etuk EU. Animal models for studying diabetes mellitus. Agric Bio J North Amer 2010; 1(2): 130-134.
24. Nnamani PO, Attama AA, Ibezim EC, Adikwu MU. SRMS 142-based solid lipid microparticles: Application in oral delivery of glibenclamide to diabetic rats. *Eur J* Pharm Biopharm 2010; 76: 68–74.
25. Barakat SN, Yassin EB. *In vitro* characterization of carbamazepine-loaded precifac lipospheres. Drug Del 2006; 13: 95–104.

26. Umeyor EC, Kenechukwu FC, Ogbonna JD, Chime SA, Attama AA. Preparation of novel solid lipid microparticles loaded with gentamicin and its evaluation *in vitro* and *in vivo*. J Micro 2012: 1- 12. DOI: 10.3109/02652048.2011.651495.
27. Brown SA, Chime SA, Attama AA, Agu C, Onunkwo GC. *In vitro* and *in vivo* characterization of piroxicam-loaded dika wax lipospheres. Trop J Pharm Res 2013; 12 (1): 33-38.