British Journal of Pharmaceutical Research
13(2): 1-10, 2016, Article no.BJPR.8168
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Novel Solid Lipid Microparticles Drug Delivery Systems for Oral Delivery of a Herbal Antidiabetic Extract

Mumuni A. Momoh[1*], Musiliu O. Adedokun[2] and Jameelu Muazu[3]

[1]Drug Delivery Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Akwa- Ibom State, Nigeria.
[3]Department of Pharmaceutics and Phamaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Maiduguri, Borno State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/8168
Editor(s):
(1) Faiyaz Shakeel, King Saud University, Riyadh, Saudi Arabia.
Reviewers:
(1) Sophia Wan-Pyo Hong, Seoul National University, Seoul, South Korea.
(2) Mohamed El Amine Dib, University of Abou Bekr Belkaïd, Algeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/16216

Original Research Article

Received 2nd December 2013
Accepted 24th February 2014
Published 19th September 2016

ABSTRACT

Aim: The aim of the present study was to prepare and evaluate a novel multiparticulate pharmaceutical formulation of *Vernonia amygdalina* extract suitable for oral administration.
Study Design: Solid lipid microparticles (SLMs) of *Vernonia amygdalina* aqueous extract and evaluate in vivo
Place and Duration of Study: Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, between October 2011-2013.
Methods: Phospholipon® 90H and Homolipid (HLP) from *Capra hircus* were used to prepare solid lipid microparticles (SLMs) of *Vernonia amygdalina* (VA). The SLMs were characterization based on size, morphology, stability and encapsulation efficiency (EE%). *In vitro* release was performed in phosphate buffer while the antidiabetic properties of the SLMs were evaluated in alloxan- induced diabetic rats.

**Corresponding author: Email: jointmomoh@yahoo.com;*

Results: There was a dose dependent increase in EE% irrespective of the type of matrixing agents used. Generally, the batches containing 400 mg VA (A1-A3) had higher EE (79.24, 81.42 and 89.16%) than those containing 200 mg VA (B1-B3; 72.19, 73.09 and 84.21%). The ratio of Phospholipon® 90H and Homolipid determined the EE% of the formulation. The formulations are stable, the particle size range 38.1 µm to 81.1. The release of VA in phosphate buffer varied widely with the lipid contents. Moreover, significant antidiabetic ($p<0.05$) properties were observed in all the VA-loaded SLM formulations.

Conclusion: The SLMs-based formulations would likely offer a more effective means of delivering *Vernonia amygdalina*, a natural extract with good antidiabetic activities as compared to the traditional way of taking the extract orally.

Keywords: Vernonia amygdalina; SLMs; antidiabetics; delivery.

1. INTRODUCTION

Diabetes is a metabolic disorder that has affected an estimated 285 million people worldwide in 2010 and the number is increasing in rural and poor populations throughout the world. It is projected to become one of the world's main disablers and killers within the next 25 years [1]. Diabetes leads to both premature death and complications such as blindness, amputations, renal and cardiovascular diseases. The disease is characterized by high blood glucose levels (hyperglycemia) due to the inability of the body's cells to utilize glucose properly, often combined with insulin resistance [2-4]. Management of this disease is largely based on two approaches: non-pharmacological approach (diet and exercise) and/or the pharmacological approach (insulin and oral hypoglycaemics). The conventional medical approach of simply using insulin and oral drugs to control diabetes mellitus is inadequate, boring and lacks compliance; thus, the patient's exposure to long term complications remains a risk [5,6].

Some wild herbs and spices have been shown to be most effective, relatively non-toxic and have substantial scientific documentation to attest to their efficacy in diabetes management [7]. In many developing countries, larger parts of the population rely heavily on traditional medicinal plants to meet their primary health care needs and also to manage their diabetes mellitus. This is due to their perceived effectiveness, minimal side effects in clinical experience and relatively low cost. In addition, resistance to multi-component preparations, as is seen in herbal medicines, develops less rapidly [8,9]. It was in this light that the World Health Assembly, in 2008, adopted among its resolutions, the support of national traditional medicine programs, drawing attention to herbal medicines as being of great importance to the health of individuals and communities [10].

Several medicinal plants, herbs, and functional foods have been found to improve blood glucose control [11], and many more are still being explored. Clinical trials have identified some plants such as *Vernonia amygdalina* as antidiabetic agents, but the pure chemical compounds isolated from the crude extracts of these plants do not bear any structural resemblance to the antidiabetic drugs in current clinical use, nor do they have similar mechanisms of action. Still, the search for novel antidiabetic drugs advocates the utilization of such plants as a potential source of such drugs [8,11].

Among these plants is *V. amygdalina. V. amygdalina* (F Asteraceae), is a small shrub that grows in the tropical Africa. It is commonly called bitter leaf because of its bitter taste. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections [12]. Many herbalists and naturopathic doctors recommend aqueous extracts for their patients as treatment for emesis, nausea, diabetes, loss of appetite-induced abrosia, dysentery and other gastrointestinal tract problems. Until the last decade or so, there were only anecdotal reports and claims to support the health benefits. The anecdotal reports are now being supported by scientific evidence that a *V. amygdalina* regimen or consumption as dietary supplements may provide multiple health benefits [6,13]. Researchers have confirmed the antidiabetic property of this plant [6,14]. The researchers concluded that water extract from

this plant has shown a considerable level of blood glucose reduction when compared to the convention drugs [6].

To our knowledge there is no report on *V. amygdalina* solid lipid microparticles (SLMs) preparations in literature, despite the numerous advantages of the SLMs in drug delivery system. Application of novel drug delivery systems such as SLMs to plant extracts minimizes the drug degradation or pre-systemic metabolism, reduces side effects and enhances patients' compliance [15]. Among modern drug delivery carriers solid lipid microparticles (SLMs) seemed to be a promising colloidal carrier system. Solid lipid microparticles made from biodegradable solid lipids exist in the micron size range and can be prepared by several methods. The advantages of SLMs include; possibility of controlled drug release and drug targeting, protection of incorporated compound against chemical degradation, no biotoxicity of the carrier, avoidance of organic solvent and no problems with respect to large scale production [16].

Thus, in this study we investigate the suitability of Phospholipon® 90H and a homolipid from *Capra hircus* as the lipid carriers for *Vernonia amygdalina* by incorporating it into solid lipid microparticles (SLMs), using standard non-toxic stabilizer such as; Sorbic acid is used as preservative and sorbitol is use as lyoprotectant.

2. MATERIALS AND METHODS

2.1 Materials

The following materials were used as procured from their local suppliers without further purification: homolipid from *Capra hircus*, Phospholipon® 90H (Phospholipid GmbH, Köln Germany), sorbitol (Across Organics, Germany), thiomersal (Synochem, Germany), PEG 4000 (Sigma, UK), methanol (BDH, UK). All other materials used were of analytical grade. Distilled water was obtained from our laboratory.

2.2 Preparation of the *Vernonia amygdalina* Extract

Pesticide-free fresh leaves of *Vernonia amygdalina* was collected at early hours daily for two days in the month of June, 2008 from Pharmacognosy garden, University of Nigeria Nsukka, and the botanical identity was confirmed by a taxonomist Mr. Ozioko of the Bioresources Development and Conservation Program (BDCP), Nsukka. The plant material was then washed with distilled water, sun dried for 5 hr daily for 5 days, and milled to get (475 g) coarse light green powder. After the milling, 450 g quantity of the powder was weighed on a mettler balance (Mettler, Toledo, BD202, USA) and used for the extraction. The weighed powder was extracted with 1.5 Litre of methanol in a Soxhlet extractor for 8 h. The methanol extract was poured into flat trays and allowed to concentrate under fan. The dried concentrate was stored in air-tight cellophane bags for further analysis.

2.3 Extraction of Homolipid from Goat Fat

About 20 kg of goat fat was processed in the laboratory as reported previously by wet rendering method [17]. Briefly, the goat fat was immersed in hot water maintained at 80 - 90°C for 45 min. A porcelain cloth was used to remove the unwanted material matter, and the fat, after cooling, was recovered by simple decantation of the lower aqueous layer. The extracted fat was further subjected to purification by passing it through a column of activated charcoal and bentonite (3:1) at 100°C at a ratio of 10 g of the fat and 1 g of the column material. The fat was stored in a refrigerator until used.

2.4 Methods

2.4.1 Preparation of *Vernonia amygdalina* (VA) loaded SLMs

The lipid matrices consisting of 1:1, 1:3 and 3:1 mixtures, respectively, of Phospholipon® 90 H and homolipid were prepared by fusion to obtain the lipid matrix [15]. Briefly, the lipid core was weighed and melted on a water bath maintained at 70-72°C. *Vernonia amygdalina* extract powder was dispersed into the molten lipid phase and stirred thoroughly. Thiomersal and sorbitol were carefully weighed and dissolved in distilled water at 75°C and immediately transferred into the lipid phase containing the extract at the same temperature. The mixture was then homogenized with a mixer (Silverson, UK) for 4 min and allowed to recrystallize at room temperature. The emulsification was further assisted by vortexing at 8000 rpm for 5 min (T 18 digital Ultra-Turrax®; IKA, Staufen, Germany). The greenish-milky dispersion was then rapidly cooled to 20°C by immersing the formulation in an ice bath without stopping the agitation to yield a uniform dispersion of SLMs. The SLMs suspension obtained after cooling at room temperature was then filter using miliport filter (0.25 µm) to get

water-free SLMs. By adding increasing concentrations of V.A (200 and 400 mg) to the lipid matrix 1:1, 1:3 and 3:1 and following the above described procedure, VA-loaded SLMs (batches A1–A3 and B1–B3 respectively) were obtained; the unloaded-SLMs (without drug) C1–C3, were similarly prepared. The formulation compositions are shown in Table 1.

2.5 Characterization of the SLMs Formulation

2.5.1 Determination of percentage yield

The percentage (%) yield of the water free SLMs was calculated using the formula:

$$\text{Percentage yield} = \frac{W1}{W2 + W3} \text{ X } 100 \qquad (1)$$

where W_1 is the weight of the lipospheres formulated (g), W_2 the weight of the drug added (g) and W_3 the weight of the lipid and the stabilizer (g).

2.6 Particle Size Analysis and Morphology

The particle size of the SLMs was determined by computerized image analysis of at least 30 lipospheres on a photomicroscope (Lieca, Germany). Each of the batches was mounted on a slide and observed under a light microscope. With the aid of Moticam 1000 camera® mounted on the microscope, the image of the particles and the corresponding diameter of the particle sizes of the SLMs were determined and average calculated. The particle morphologies were also observed and photomicrographs taken. All these were done in a time-dependent manner (24 h, 1 week, and 8 weeks).

2.7 Encapsulation Efficiency

The entrapment efficiency (EE) of the prepared SLMs was assessed using Vivaspin ultra-centrifuge tubes (Vivaspin® 6 Centrifugal Concentrator, molecular weight cutoff [MWCO] 100,000; Viva products, Inc, Littleton, MA). SLMs dispersion (3.0 mL) was added to the centrifuge tube and centrifugation was carried out for 15 minutes at 4000 rpm. The supernatants were adequately analyzed by UV/Vis spectrophotometer (Unico 2102, England) at 235 nm. The amount of drug encapsulated in the SLMs was calculated to obtain the % encapsulation efficiency (EE) using the formula below:

$$EE\ \% = \frac{Mi - Mf}{Mi} \quad X\ 100 \qquad (2)$$

Where Mi is the initial weight of VA extract and Mf is the weight of the free (unentrapped) VA detected in the filtrate.

2.8 *In vitro* Drug Release Studies

Dialysis method was used to determine the release of VA from the SLMs. The polycarbonate dialysis membrane used was pre-treated by soaking it in the dissolution medium for 24 h prior to the commencement of each release experiment. In each case, 0.2 g of the formulated SLMs was placed in the dialysis bags with a molecular weight cut-off of 50,000 Da (Sigma-Aldrich) containing 2.0 mL of 50 mL phosphate buffered of pH 7.2 in a 250 mL beaker, securely tied with a thermo-resistant thread and then submerged in the dissolution medium under agitation provided by the magnetic stirrer at 100 rpm and maintain at 37℃. At predetermined time intervals, a 5 mL portion of the dissolution medium were withdrawn and was replaced with equivalent volume of the same medium to maintain sink condition throughout the experiment. Each sample withdrawn was filtered and analyzed spectrophotometrically (Unico 2102, England) at 235 nm.

2.9 *In vivo* Anti-hyperglycemic Assessment

2.9.1 Induction of diabetes

Fourty male Wister rats weighing between 180 and 200 g were used in this study. The animals were purchased from the animal utility house of the Department of Pharmacology, University of Nigeria Nsukka. Rats housed in cages were kept in a room with controlled temperature (20–22℃) and a 12 h day–night cycle. 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. A volume equivalent to 1 ml of the stock solution was given intra-peritoneally after which the blood glucose levels were measured frequently for 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. Before the commencement of the experimental work, the protocol on the use of animal was approved by the Ethical Committee of Department of Pharmaceutics, University of Nigeria Nsukka.

2.10 Protocol for Administration of VA-Loaded SLMs

Fourty diabetic rats were randomly divided into eight groups of five rats per group and housed in a separate cage. Group 1 received conventional metformin hydrochloride (MTH) marketed as glucophage® 500 mg/kg, and served as positive control; the group 2 received unloaded SLMs C1, and served as negative control. Group 3 4 and 5 received formulation A1-A3 while group 6 through 8 received formulation B1-B3 respectively. All the administrations were done orally.

2.11 Assessment of the Anti-hyperglycemic Effect of Oral VA on Diabetic Rats

Prior to commencement of the studies the animals were fasted for 16 h and blood glucose concentration was determined using a glucometer (Accu-check, Roche, USA). Blood glucose values of 120 mg/dl and above were considered diabetic. The diabetic rats were then treated according to the protocol and blood glucose concentrations determined at predetermined times up to 16 h.

2.11.1 LD_{50} experiment

Toxicity of the SLMs formulation was also studied by LD_{50} experiment. Three groups of albino male and female mice (n = 5) weighing about 18–21 g were used for the determination of toxicity study. They were orally administered a dose of lipospheres formulation equivalent to 2000, 3000 and 4000 g/kg of *V. amygdalina* respectively, to represent ten to twenty times of the most effective therapeutic dose observed in this study. This method was chosen to enable us assess the effect of overdose and also to established the limit of consumption. Parameters such as gross behavioral, neurologic, autonomic and toxic effects were observed for a period of 48 h. Things like intakes of food and water consumption were similarly observed for a period of 12 h.

2.12 Statistical Analysis

Data presented in this paper are expressed as the means ±S.E.M. Statistical comparisons between groups were made either by a Student's t test or by a one-way analysis of variance, followed by Fisher (least significant difference) multiple comparison procedure to assess significant differences between groups with $p < 0.05$ being considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Percentage Recovery of SLMs

The result presented in Table 2 was obtained for the SLMs formulated. The percentage recovery was very high. This may be as a result of adoption of a reliable production process technology with minimal loss.

Representative of the photomicrographs of the loaded and drug-free SLMs prepared with homolipid and P90H® are presented in Fig. 1. The morphologyical features of the various

Table 1. Quantities of materials used for the SLMs formulations

Batch	P90H : HLP	Thiomersal (% w/w)	VA (mg)	Sorbitol (% w/w)	Distilled water (% w/w)
A1	1:1	0.01	400	2.0	100
A2	1:3	0.01	400	2.0	100
A3	3:1	0.01	400	2.0	100
B1	1:1	0.01	200	2.0	100
B2	1:3	0.01	200	2.0	100
B3	3:1	0.01	200	2.0	100
C1	1:1	0.01	0.00	2.0	100
C2	1:3	0.01	0.00	2.0	100
C3	3:1	0.01	0.00	2.0	100

Note: P90H= Phospholipon® 90H; HLP = homolipid; VA= Vernonia amygdalina

Table 2. Properties of the SLMs loaded with increasing concentrations of *Vernonia amygdalina* extract

Formulation parameters				Time resolved particle size analysis		
Batch	**P90H : HLP**	**EE%**	**YD %**	**48 h**	**4 weeks**	**8 week**
A1	1:1	79.24 ± 0.20	89.1±0.11	51.2± 13.0	68.9± 22.1	81.1 ± 1.33
A2	1:3	81.42 ± 0.05	92.3 ± 0.11	47.4± 9.10	53.1± 20.2	65.7 ± 0.31
A3	3:1	89.16 ± 0.24	96.0 ± 1.20	55.0± 11.0	53.4± 34.6	79.7 ± 0.12
B1	1:1	72.19 ± 1.20	83.1 ± 3.10	38.1± 0.06	41.0± 0.91	68.1 ± 0.98
B2	1:3	73.09 ± 0.10	89.9 ± 0.20	49.1± 0.80	46.8± 0.54	59.1 ± 1.11
B3	3:1	84.21 ± 0.44	92.7 ± 0.41	43.0± 8.70	48.9± 16.1	61.1 ±1.00
C1	1:1	--	76.3 ± 0.2 1	33.1± 73.0	39.6± 90.1	31.1 ±1.33
C2	1:3	---	87.1 ± 3.12	32.7± 10.0	32.1± 12.1	37.8 ±1.03
C3	3:1	---	75.2 ± 2.90	35.0± 10.0	31.2± 24.1	45.1 ±0.74

Note: P90H= Phospholipon® 90H; HLP = homolipid. EE %= Encapsulation efficiency, YD = percentage yield

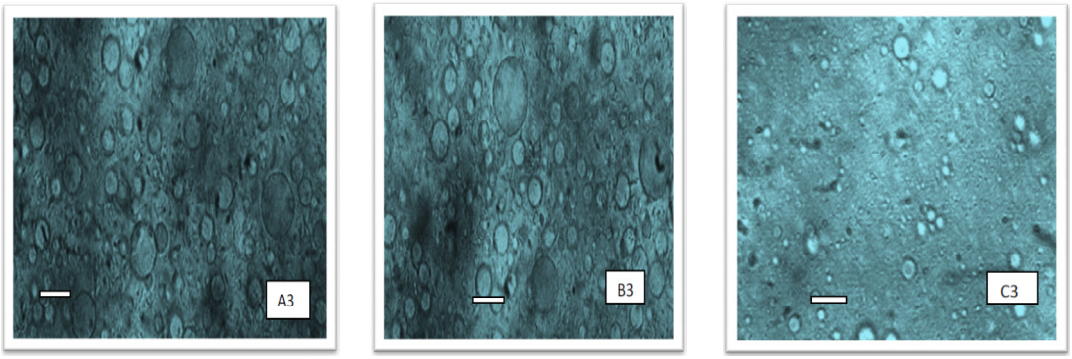

Fig. 1. Photomicrographs of representative batches of SLMs s containing P90H: HLP after 8 weeks: A3, B3 and C3 contain 400, 200 and 0 mg of *Vernonia amygdalin* extract, respectively. Bar represents 65 µm

batches of the SLMs showed an irregular shaped. However, the formulation remained stable in suspension form after storage for 8 weeks. The loaded and drug-free SLMs showed similar morphological features, indicating that the incorporation of *V. amygdalina* extract into the lipospheres did not significantly (p> 0.005) alter the shape of the particles. The reason for the irregular shaped SLMs is uncertain, but may be due to preparation of the SLMs without a lyoprotectant or could be due to some technical problems resulting from the preparation. This may be related to the solubilities of the different components of the extract in the homolipid. The lipophilic components of the extract is expected to form a continuous structure with the homolipid unlike the water soluble components, which may form partly soluble mixture and partly suspended non-coherent structure resulting in irregularly formed structures. The possible effect may be that SLMs with larger particle sizes will possess lower rate of emulsification *in vivo*, than the SLMs with smaller particle sizes and consequently, larger emulsion droplets may be formed as a result.

3.2 Physicochemical Properties of SLMs

The particle size and entrapment efficiency of the SLMs formulation are presented in Fig. 2 and Table 2. The results showed that the particle size of unloaded SLMs (C1-C3) were in the range of 35.0 ± 10 to 45.1 ± 0.74 µm. This was slightly less than the particle size range of VA-loaded lipospheres (47.4 ± 9.1 to 81.1 ± 1.3 µm) and (38.1 ± 0.06 to 68.1 ± 0.98 µm) for sub-batches in A and B respectively. In all the formulations, sub-batches A1, B1 and C1 possessed the lowest mean particle sizes when compared to other batches. However, when sub-batches A1, B1 and C1 were compared, it was observed that B1 posssessed the least mean particle size. There was slight increase in particle size between 24 h and 8 weeks for SLMs from all the batches containing varying quantities of lipids and the *VA* (Table 2). Increase in particle size is

usually as a result of aggregation and subsequent growth by Ostwald ripening or sintering. However, the apparent particle size stability obtained after 8 weeks for the SLMs formulated based on this method could be adjudged to be relatively stable. The least particle sizes of the unloaded and *VA*-loaded SLMs as determined by photon correlation spectroscopy were found to be 32.0 and 38.0 µm respectively within 24 h of the formulation. The particle sizes increased to 45.0 and 81.0 µm respectively, upon storage for 8 weeks, which may be due to crystallization of the formerly molten matrices. The growth of the particles did not affect their shapes (Fig. 2).

3.3 Encapsulation Efficiency (%)

The VA-loading efficiency increased with increase in the concentration of the VA such that the maximum and minimum encapsulation efficiency (%) of *VA* are 89.10 (SLM-A3 containing 400 mg of VA and lipid mixture of 3:1 of P90H and homolipid) and 73.09 (SLM-B2 containing 200 mg of VA) as shown in Table 1. It shows that the lipid matrix promoted concentration-dependent *VA* encapsulation. The lipid matrix accommodated more *VA* at higher *VA*-loadings due to the low crystalline nature of the excipients. However, the formulation showing higher EE (%) can be evaluated for further use. More so, the result here showed that the lipid matrices have the capacity to be used as carriers for natural compounds such as *Vernonia amygdalina* extract as demonstrated in this work. The therapeutic efficacy of any molecule or compound depends largely on the delivery system and the ability of the carrier(s) to accommodate more of the active agents and also to make it available for better therapeutic value. The clinical implication of this result is that the therapeutic agent (VA) can be encapsulated in these carriers in greater quantity for effective release in the biological medium for optimum absorption.

3.4 *In vitro* Release Study

The *in vitro* release profiles of VA in phosphate buffer indicate very significant ($p < 0.005$) release of VA from all batches of the formulation as shown in Fig. 2. The results reveal that the release of VA from the SLMs is dependent on the particle sizes of the SLMs. The highest release (92%) and (87%) were found to be in formulation A3 and B3 respectively; these were attained at 60 min. Formulation A1 and B1 gave maximum release of 73% and 69% respectively in 60 min, while that of A2 and B2 were 55% and 50% respectively at 45 min. One interesting thing in the release of the VA is that the release depends largely on the P90H present in the formulation. It also shows that the higher the particles size the more the release. Regarding their sustaining potentials, all the batches of the SLMs have good sustaining properties. However, batch A3

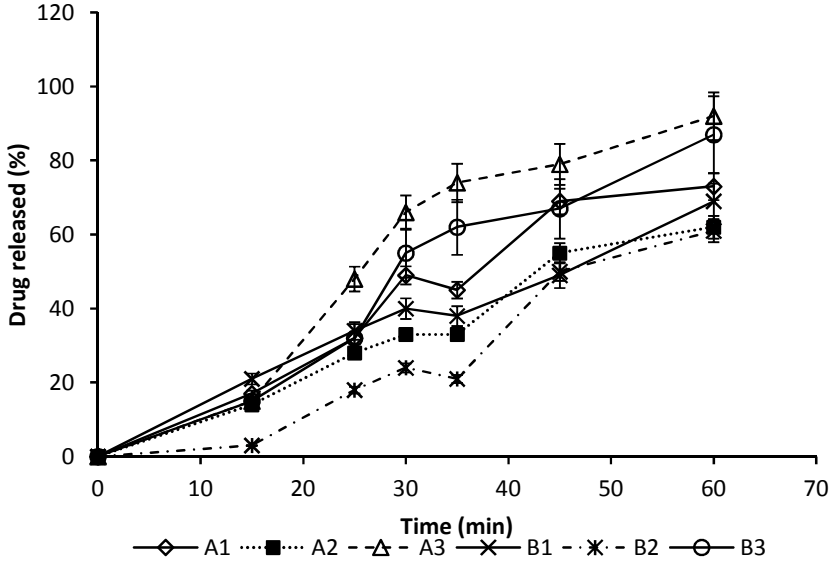

Fig. 2. *In vitro* release profile of VA from SLMs formulated with P90H:HLP of 1:1, 1:2 and 2:1 in phosphate buffer system. A1–A3 contain 400 mg of VA and B1–B3 contain 200 mg of VA

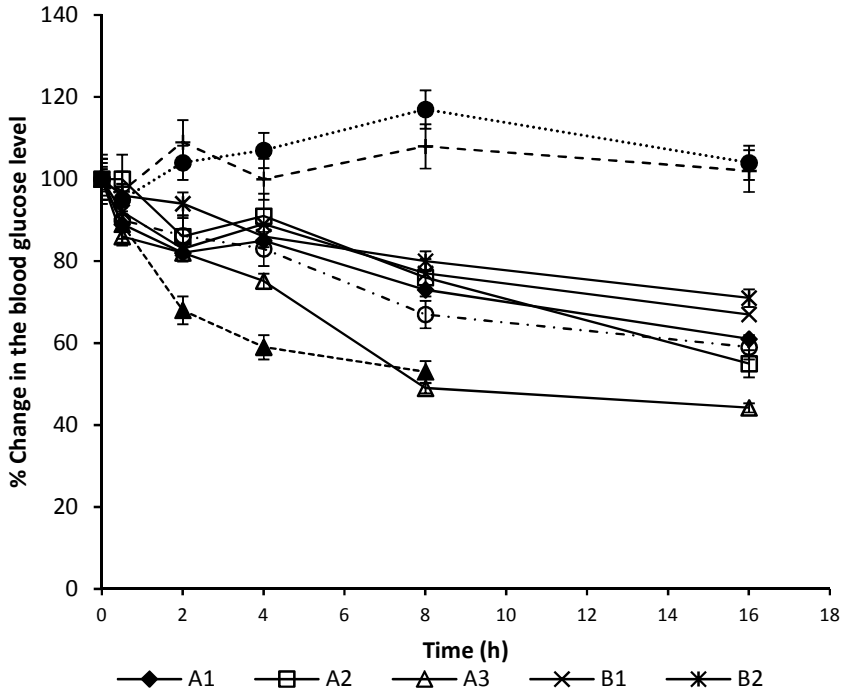

Fig. 3. Antidiabetic profile of SLMs loaded with *Vernonia amygdalina* extract (*n*= 3). A1–A3 contain 400 mg of VA and B1-B3 contain 200 mg of VA respectively, while DW, MTH and C1 represent distilled water, conventional metformin hydrochloride (MTH) (which serve as positive control) and unloaded SLMs respectively

formulations produced better sustained release properties than batches B3 since it released and maintained a uniform trend through the period of the study. The release trend here could be said to be the desired pattern in the management of diabetic disease. The initial burst observed in this batch A3, may be due to the VA that attached or adsorbed to the surface of the lipid matrix and not within the core lipid [8]. The clinical implication is that in acute case of diabetes, the formulation could be a cheaper alternative to oral hyperglycemic agents.

3.5 Pharmacodynamics *In vivo*

Fig. 3 shows the behavior of different formulations administered orally to diabetic rats. The efficacy of the formulation was assessed by measuring the blood glucose concentration and calculating the relative pharmacological availability. All the groups that received *VA*-loaded SLMs showed blood glucose lowering effect in alloxan induced diabetic rats. SLMs formulations containing 400 mg of VA (batches A1-A3) showed a stronger effect on blood glucose levels when compared with batch B1-B3 which contained 200 mg of VA. There was no such reduction in the rats groups that received batch C (unloaded SLMs). All the formulations in batches A and B exhibited good glucose reduction level compared to MTH (positive control). However, similar effect was observed when the extract was in a traditional method but, less potent and with short duration of activity in diabetic rats compared to SLMs formulations [14, 15]. The results obtained in this study strongly provide scientific evidence to support the ready availability of natural biomolecules from properly designed and prepared pharmaceutical formulations. Our results also show that, the technology adopted produced highly reproducible formulations as against the traditional method that lack reproducibility and stability [14]. The mechanisms through which this extract performed its antidiabetic action has been discussed by previous researchers [14,15]. The observation that *V. amygdalina* loaded SLMs reduced blood glucose in the diabetic rats implies that the anti-hyperglycemia principle(s) in *V. amygdalina* may have direct effect comparably

to the conventional formulation. The bioactive molecules present in indigenous vegetables may possibly possess insulin-like effect or stimulate the pancreatic beta cells to produce insulin which in turn lowers the blood glucose level [18]. Similar observations have been reported by other researchers [19] on the same unformulated plant. Our findings revealed that administration of formulated *V. amygdalina* loaded SLMs showed a significant dose- dependent reduction of blood glucose levels in normal rats. This result was in agreement with study carried out using unformulated extract by Gupta et al. [20], indicating, that the preparation technique used in the SLMs formulation did not alter or denature the active components of the extract and was able to delivered the active component effectively.

3.6 Safety Studies

Pharmaceutically, investigation of the acute toxicity is the first step in the toxicological study of an unknown substance intending to be used in the management, diagnosis or prevention of disease or for animal or human consumption. Although, *V. amygdalina* has a very long history of use as a vegetable in soups in Nigeria, it is paramount to assess its level of safety before it can become scientifically approved to be used in the management of diseases. Based on the doses tested on each rat, there was no sign of any toxicity or death recorded throughout the monitoring period. All the rats in each group appeared normal, active and responsive to any external stimulus. Water intake and food consumption when compared to the control showed no difference. The oral LD_{50} of the extract was estimated to be greater than 4,000 mg/kg body weight. This extract may be safe for human consumption, as the LD_{50} was determined to be greater than 4 g/kg body weight.

4. CONCLUSION

This study demonstrates that microscopic SLMs have attractive properties, such as stability of the preparation, high encapsulation efficiency, and high release, which invariably improves its bioavailability and enhances its antidiabetic properties after oral administration. LD_{50} evaluations revealed that VA-loaded SLMs did not show any sign of toxicity as the formulations were well tolerated at concentrations tested. Compared to positive control, the formulation showed a stronger blood glucose reduction. It is concluded that this delivery system for VA may bring us closer to a safe, general system for oral administration of this natural antidiabetic compound.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed, as well as specific national laws where applicable. In accordance with the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka.

ACKNOWLEDGEMENTS

The authors expressed their gratitude to Dr. J.O Ezugworie of the Department of Anatomy, University of Nigeria Teaching Hospital Enugu Campus for providing the necessary facilities for carrying out the diabetes study. And to Dr. A.T Mora for reading and making appropriate corrections on the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shaw JE, Sicree RA, Zimment PZ. Global estimates of the prevalence of diabetes for (2010) and 2030. Diabetes Res. Clin. Pract. 2010;87:4–14.
2. Ashwani G, Sandeep K, Manju N, Inderbir S, Sandeep A. Potential of novel drug delivery systems for herbal drugs. Ind J Pharm Edu Res. 2011;45:225-235.
3. Wild S, Sicree R, Roglic G, King H, Green A. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–1053.
4. Philip AK, Srivastava M, Pathak K. Buccoadhesive gels of glibenclamide: A means for achieving enhanced bioavailability. Drug Deliv. 2009;16:405–415
5. Adeneye AA, Agbaje EO. Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaves of ethanol extract of *Morinda lucida* Benth in

normal and alloxan-induced diabetic rats. Afr J Biomed. 2008;11:65–71.
6. Okolie UV, Okeke CE, Oli JM, et al. Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. Afr J Biotech. 2008;7:4581–4585.
7. Okeke EC. The use and chemical content of some indigenous Nigerian spices. J. Herbs Spices Med. Plants. 1998;5:51-63.
8. Emeje MO, Izuka A, Isimi CY, Ofoefule SI, Kunle OO. Preparation and standardization of a herbal agent for the therapeutic management of asthma. Pharm Dev Tech. 2011;16:170–178.
9. Biswakanth KRB, Suresh K, Asis B, Narayan D, Upal KM, Pallab KH. Evaluation of antitumor activity of *Mimusops elengi* leaves on ehrlich's ascites carcinoma-treated mice. J Dietary Suppl. 2012;9:166–177.
10. World Health Organization. Traditional medicine. Fact sheet No. 134; 2008. Available:http://www.who.int/mediacentre/factsheets/fs134/en/
11. Rout SR, Chowdary KA, Kar DM, Das L. Plants as source of novel anti diabetic drug: Present scenario and future prospective. Curr Trends Biotechnol Pharm. 2009;3:37–55.
12. Chan ES, Yim ZH, Phan SH, Mansa RF, Ravindra P. Encapsulation of herbal aqueous extract through absorption with ca-alginate hydrogel beads. Food and Bioproducts Processing. 2010;88:195-201.
13. Uliyar VM, Indirani M, Mamta B, Smriti NK. An open-label study on the effect of flax seed powder (*Linum usitatissimum*) supplementation in the management of diabetes mellitus. Journal of Dietary Supplements. 2011;8:257–265.
14. Akah P, Okafor C. Blood sugar lowering effect of *Vernonia amygdalina* in an experimental rabbit model. Phytother. Res. 1992;6:171-173.
15. Momoh MA, Akpa PA, Attama AA. Phospholipon 90G based SLMs loaded with ibuprofen: An oral anti-inflammatory and gastrointestinal sparing evaluation in rats. Pakistan J. Zool. 2012;44:1657-1664.
16. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomed. 1998;2:137–189.
17. Anthony AA, Megg ON. *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.
18. Uchenna VO, Chinwe EO, John MO, Ijeoma OE. Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. Afr. J. Biotech. 2008;7:4581-4585.
19. Fuentes O, Arancibis A, Alarcon H. Hypoglycemic activity of *Bauhinia candican* in diabetic induced rabbits. Fitoterapis. 2004;6:527-532.
20. Gupta S, Mal M, Plaban B. Evaluation of hypoglycemic potential of *Solanum anthocarpum* (solanaceae) fruits in normal and streptozotocin- induced diabetic rats. Eur. Bull. Drug Res. 2005;13:51-55.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16216