Chinese Herbal Medicines 12 (2020) 142–155

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Role of semi-purified andrographolide from *Andrographis paniculata* extract as nano-phytovesicular carrier for enhancing oral absorption and hypoglycemic activity

Vinod Kumar Verma[a,b], Md. Kamaruz Zaman[a], Shekhar Verma[c,*], Santosh Kumar Verma[d,e], Khomendra Kumar Sarwa[f]

[a] *Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, India*
[b] *State Food and Drug Testing Laboratory, Raipur 492001, India*
[c] *University College of Pharmacy, Pt. Deendayal Upadhyay Health, Science and Aayush University Raipur-492001, Chhattisgarh, India*
[d] *School of Chemistry and Chemical Engineering, Yulin University, Yulin 719000, China*
[e] *Shaanxi Key Laboratory of Low Metamorphic Coal Clean Utilization, Yulin University, Yulin 719000, China*
[f] *Department of Pharmacy, Government Girl's Polytechnic, Raipur 492001, India*

ARTICLE INFO

Article history:
Received 25 June 2019
Revised 23 September 2019
Accepted 10 October 2019
Available online 25 December 2019

Keywords:
Andrographis paniculata (Burm. f.) Nees
andrographolide
hypoglycemic activity
liquid dispersion
nano-phyto vesicle
soya phosphatidylcholine

ABSTRACT

Objective: *Andrographis paniculata* is a well-known medicinal plant in Southeast Asia, India and China. The plant contains andrographolide (AN), a very important phytochemical used in various health problems. However, AN is low in oral absorption bioavailability of AN due to the rapid clearance and high protein binding capacity.

Methods: The present study was aimed to develop a nano-phytovesicular formulation of semi-purified AN extracts from a naturally occurring phospholipid (soya phosphatidylcholine) in order to increase the oral absorption and antihyperglycemic activity in rats.

Results: The nano-phyto vesicle of semi-purified AN extracts equivalent to 25 mg /kg AN significantly protected the hyperglycemic condition of rats. The *in vitro* and *in vivo* experiments results proved that the nano- phytovesicular system of plant extracts containing AN produced better oral absorption, bioavailability and improved antihyperglycemic activity compared with that of free AN at dose of 50 mg/kg.

Conclusion: Hence, the prepared semi-purified extract nano-phytovesicular system is helpful in solving the problem of rapid clearance of AN.

1. Introduction

Diabetes mellitus or diabetes is a disease of pancreas. It is a chronic disease caused by deficiency in insulin production by the pancreas, or by the inefficacy of the insulin produced. The deficiency in insulin production and inefficacy results in increased amount of blood glucose level, which injurious for many body's physiological process. It may produce negative and harmful impact on excretory system, circulatory system and nervous system. All type II diabetes mellitus is characterized due to the metabolic defects and causing insulin resistance. Variety of oral hypoglycemic agents is available in market including insulin but there is an increased demand of natural herbal products by the patients due to minimum side effects (Chinsembu, 2019; Zhang et al., 2017). Medicines from natural plant origins are widely used throughout the world for variety of insulin deficiency complications. Nevertheless, natural plant drugs have no suitable delivery system to achieve better bioavailability. Therefore, pharmacokinetic profile of active constituents if enclosed in the carrier forms and novel carrier systems of plant extracts has more important and somehow it is more challenging area for further research.

Andrographis paniculata (Burm. f.) Nees (Acanthaceae), a conventional herb with many effective medicinal properties, has lot of ethnobotanical evidence in India and China. It is a popular

* Corresponding author.
E-mail addresses: shekharpharma@gmail.com (S. Verma), vermasantosh08@yulinu.edu.cn (S.K. Verma).

https://doi.org/10.1016/j.chmed.2019.12.004

medicinal plant used from the ancient time. *A. paniculata* is also popular as Kalmegh and widely used as an effective medicinal plant. It has common ingredient of 40 polyherbal formulations considered in India and used in different system of medicine included Ayurveda and Unani (Chadha, 1985). It is also used as a bitter tonic for the treatment of variety of hepatic disorders (Brahmachari, 2017; Zhang, Sun & Wang, 2013).

The traditional medicinal plants of *A. paniculata* and its derived drugs are the potential source of alternative medicine and are tremendously used to treat various health ailments related to GIT and digestion, vermicide, anticancer, analgesic, anti-inflammatory, antibacterial, antityphoid, antihyperglycemic and liver impairment (Bhan et al., 2006; Matsud et al., 1994; Murugian, Palanisamy, Stanley & Akbarsha, 1995; Saxena et al., 2000; Sheeja, Guruvayoorappan & Kuttan, 2007; Verma, Sarwa & Zaman, 2013). It is also used traditionally for treatment of fever, dysentery, dyspepsia, relief of general debility, cancer, general and severe inflammation, common cold, tonsillitis, throat infection, lungs infection, and hepatic destruction (Ahmad, Ahmad, Arshad & Afzal, 2014; Bombardelli, 1994; Widyawaruyanti et al., 2014). An anti-HIV activity concluded from Indonesia of the crude whole plant extract (Otake, Mori, Morimoto, Hattori & Namba, 1995) and is also reported on the management of respiratory tract infection (Yu, Hung, Chen & Cheng, 2003). The main active constituent of the leaves of *A. paniculata* and ethanol extract of *A. paniculata* decreases blood sugar level in both normal and diabetic rats (Shen, Chen & Chiou, 2002; Zhang &Tan, 2000). The medicinal values and effectives of these herbs are mainly due to diterpene lacton and few other diterpenes, *i.e.* deoxyandrographolide and 14-deoxy-11, 12 didehydroandrographolide present in plant claimed by various researches. Diterpenoid lactone has many pharmacological effects like anticancer (Gupta, Ashawat, Saraf & Saraf, 2007; Rajagopal, Kumar, Deevi, Satyanarayana & Rajagopalan, 2003), immuno stimulation (Panossian et al., 200), antifertility (Akbarsha & Murugain, 2000; Madav, Tripathi & Mishra, 1995). anti-HIV (Calabrese et al., 2000; Maiti, Mukherjee, Gantait, Saha & Mukherjee, 2006), cardio protective (Maiti et al., 2006) and choleretic activities (Puri, Saxena, Saxena & Saxena, 1993).The AN is chemically 3-[2-{*decahydro*-6–*hydroxy*-5-(*hydroxymethyl*)–5,8*a*-*dimethyl*-2-*methylene*-1-*naphtha*-*lenyl*}*ethylidene*]dihydro-4–hydroxy, 2(3H)-furanone (Fig. 1).

Modern pharmacological studies indicate that phytoconstituents used as antihyperglycemic agent, the mechanisms by which they exert their effects are different; They can be α-glucosidase inhibitors, aldose reductase inhibitors and insulin secretagogues. They can also act by enhancing glucose uptake, increasing insulin sensitivity and inhibiting TNF-α production by macrophages. Yu et al., 2003 found that AN exerted an antihyperglycemic effects in streptozocin-induced diabetic rats. Mechanism of AN was investigated mainly as α-glucosidase inhibitor (Jung et al., 2006; Suo, Zhang & Wang, 2007; Xu, Dai, Lui, Wang & Liu, 2007).

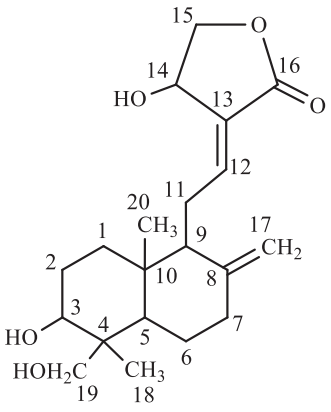

Fig. 1. Chemical structure of andrographolide.

The poor aqueous solubility, low oral bioavailability and high elimination rate are the main drawback to use single moiety for various pharmacological potential. Therefore, several strategies have been tried to overcome and improve the aqueous solubility, oral absorption and sustain release of AN, such as preparation and utilization of liposome (Suo et al., 2007), chemical modification (Ren et al., 2009), particle size reduction (Qiu, Li, Li, Zhang, & Chen, 2004) and complexation (Bothiraja, Shinde, Rajalakshmi & Pawar, 2009aa; Xue, 2003; Zhao, Liao, Ma & Yan, 2002) were aimed in AN loaded solid lipid nanoparticles (Bothiraja, Pawar, Shaikh & Sher, 2009b; Parveena, Ahmada, Iqbala, Samimb & Ahmad, 2014). A novel carrier system is needed to maintain AN concentration in blood for an extended period.

However, the problem to use AN clinically still have challenge and had not been all solved. Suitable drug carrier systems for increasing the oral bioavailability of the poorly water-soluble AN remain great interest to find. There are various phyto-constituents having poor water solubility and poor oral bioavailability like silybin, quercetin curcumin, ellagic acid, andrograholide and naringenin which having various important pharmacological activity. Researchers found that soya lecithin containing phospholipids maintained the concentration of the poor water soluble and low bioavailable drug in plasma for long duration and enhanced therapeutic efficacy. Researchers had been also reported that molecules have poor oral absorption, such molecule exerted better therapeutic efficacy if they can be converted as phospholipid complexes or vesicular carrier systems rather than the free molecules reported by Arien, Goigoux, Baquey and Dupuy, (1993) and Arien and Dupuy (1997). Phospholipids plays vital role for cell membranes and it is an important component which keep the membrane fluid consistency.

Therefore, in the present study, we try to take the advantages of phospholipids and semi-purified AN extract combined with phospholipids to nano-phytovesicular carrier systems, which might result in increase of oral absorption, bioavailability and improvement of the biological effect of AN. The specific characteristics of plant purified to extract used have many active ingredients as compare to one compound AN and these groups of active ingredients show synergistic effect and enhance the activity of the formulation.

2. Materials and methods

2.1. Materials

The soya phosphatidylcholine (SPC) (Phospholipon 90) was gifted from global phospholipids supplier-Lipoid (Germany). Pure AN was received from Cayman-Genetix Biotech Asia Pvt. Ltd. (Delhi, India). S.D. Fine Chemicals, Mumbai, India provided methanol, ethyl acetate, sodium carboxymethyl cellulose (Na CMC), sodium dodecylsulfate, *n*-hexane and *n*-octanol. HPLC grade solvents were used for chromatographic peaks, the double distilled water was purified by millipurified system for experiments. All other reagents were of analytical grade.

2.2. Authentication of plant material

Andrographis paniculata plant was collected in Dibrugarh district India and authenticated at Haarita Ayurvedic Hospital, Dibrugarh, Assam, India. Following test were performed for authentication of plant, where voucher specimens were deposited. Microphotographs of sections and powder analysis were made by using

Olympus Micro-scope (Model CX 41; Tokyo, Japan) with CCD camera 2 mega pixel and quantitative measurements were taken using Olympus Image-Pro-Plus, version 5.1 software. Fine hand sections of lamina, petiole, stem and root, and epidermal peels were taken using standard procedures and were stained with Aqueous safranin 1% and mounted in glycerin. Fluorescence analysis of the powder was carried out in UV light (256 nm & 366 nm) using Camang UV apparatus. The descriptive terms of the anatomical various plant parts are supplemented with micrographs wherever necessary and magnifications are indicated by the scale-bars in the figures. The number of epidermal cells, stomatal number, and stomatal index was calculated per square millimeter of leaf area from intercostal areas of fresh leaves and vein islet number and vein termination 1 g of *A. paniculata* powder with 20 mL of ethanol was boiled in a water bath for 5 min. A toal of 300 mg of activated charcoal was added, stirred and then filtered. The filtrate was used for the following two tests: **Test (1):** The first test involved 2 drops of a 2% (w/v) solution of 3,5-dinitrobenzoic acid in ethanol and 2 drops of a 5.7% (w/v) solution of potassium hydroxide in ethanol being added to 0.5 mL of filtrate. A colour change to a purplish red indicated that the sample had active compounds. **Test (2):** The second test involved 3 – 5 drops of the 5.7% (w/v) solution of potassium hydroxide in ethanol being added to 0.5 mL of filtrate until a red colour appeared. The filtrate was set aside for 10 to 15 min. The red colour changed to yellow. These two tests indicate that the sample contained diterpene lactones as active constituents.

Confirmatory test: To confirm that above obtained diterpene lactones obtained from *A. paniculata* is AN, the above filtrate was evaporated under reduced pressure until fully dried. About 5 mg of filtrate powder was dissolved in 5 mL of warm ethanol and 1 mg of standard AN was dissolved in 0.5 mL of warm ethanol separately. A pre coated plate of silica gel 60 F254 aluminum sheets (10 × 10 cm) was used and the mobile phase used was chloroform: methanol: ethyl acetate (8:1.5:1). Five micro liters was used for each spot. TLC of the isolated samples was firstly detected by UV radiation (Electronic UV Trans illuminator, Quantum Scientific) and then confirmed by spraying with 2% (w/v) solution of 3,5-dinitrobenzoic acid in ethanol and an excess of 5.7% (w/v) of potassium hydroxide in ethanol. Results completely match with standard AN which clearly indicates that the obtained diterpene lactones was AN.

2.3. Extraction of plant

2.3.1. Solid-liquid extraction

Method of extraction for AN includes extraction of the leaf powder by cold maceration. In the first set of experiments, the extraction was performed using different solvents. The suitable solvent which gives higher AN enrichment in the extracted phase was selected for further experiments. The leaves were ground to a powder (80 mesh size) and extracted at reflux temperature for 3 h. The extraction of AN from the ground powder was carried by mixing powder and solvent using Sox-let extraction technique. The powder to the different solvent ratio (w/v) used for all the studies. The solvent was removed and the process was repeated for one more time to remove the final traces of AN from the ground powder of leaves. The extracts were then combined and concentrated by recovering the solvent using Buchi rotavapour. The obtained brownish enriched extract of AN was used for further studies.

The airy parts of *A. paniculata* were collected from Guwahati, Assam in Aug-Sep 2010. The dried plant parts were successively extracted from different solvent such as petroleum ether, ethyl acetate and methanol. Methanol extracts was kept at (25 ± 2) °C for 3 d and following filtration; Methanol extract was vaporized in a low pressure to get sticky mass (Nugroho et al., 2012). The obtained semi-purified AN extract was kept in deep freezers at −30 °C for 24 h and vacuum dry at 40 psi in lyophilizer for 12 h. The dried semi-purified AN extract was kept in airtight container for analysis of various physicochemical parameters DSC, FTIR, TLC, HPLC and preparation of formulation.

2.4. Identification and quantification of AN in semi-purified extract

2.4.1. Percentage yield of methanolic extract

The effect of different solvents such as petroleum ether, ethyl acetate and methanol was studied. The yields (%) of *A. paniculata* with different solvents were investigated. Methanol extraction gives higher yield compared to other solvents. Different solvent extraction using methanol, petroleum ether, ethyl acetate was all performed. HPLC chromatograms of the extracts, where represented as main andrograopholide. As for the extracts, the purity of the product is the prime characteristic and quality factor. It would be easy to judge extraction methods if the target compound to be enriched was single and well-defined. In comparison to non–polar solvents, polar solvents could extract AN at higher yield except water, where hydrolysis and thermal degradation might occur. Methanol was found to be the best solvent for the extraction of AN (Mishra & Gaikar, 2006; Wongkittipong, Prat, Damronglerd & Gourdon, 2004).

2.4.2. Determination of solubility

The solubility of AN extract in methanol was measured at different temperatures. The excess quantity of AN was added to the 10 mL of solvent. Subsequently, the liquid-solid suspension was constantly agitated at 120 rpm at 30 °C for 2 h in REMI Shaker to achieve uniform mixing. The clear solution was then removed using syringe filter and dried in the vacuum oven at 50 °C. The obtained solids were weighed and the solubility was reported as mg of AN per mL of solvent. The same procedure was repeated at different temperatures in order to get solubility curve.

The solubility remarkably increases from 15 °C to 65 °C, almost by a factor of two. Before 45 °C and post 65 °C, the change in the solubility is small as compared to 15–65 °C temperature range. Moreover, the solubility profile represents an equilibrium relationship in crystallization processes. Equilibrium is reached when the solution is saturated and the equilibrium relationship indicates the significant crystallization point, where maximum recovery of crystallized product was obtained.

Solvent extract was subjected to thin layer chromatography (TLC) as per standard one dimensional ascending method. The methanol AN extract yielded maximum spots in TLC, followed by ethyl acetate and petroleum ether extracts respectively. All of these TLC profiles may serve as characteristic fingerprint of AN extract. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions.

2.4.3. Determination of λ_{max}

The semi-purified extract from *A. paniculata* containing AN and pure AN was separately dissolved in methanol, scanned in UV–vis Spectrophotometer (Shimadzu, UV-1800 Shimadzu Tokyo, Japan) using methanol as blank.

2.4.4. Determination of active compound by TLC

Thin layer chromatography of semi-purified plant extracts and pure AN was performed by using solvent system (7:1 ratio of chloroform and methanol). Sulphuric acid 4%–20% in methanol was used as detecting reagent. The Rf value of pure AN was calculated and compared with semi-purified AN extract (Mukherjee, 2002).

Table 1
Factorial design selected variable ranges of nano-phyto vesicles formulation.

Variables		Levels		
		−1	0	1
Factors (Independent variables)	X_1: Conc. of SPC (mg)	100	200	300
	X_2: Vol. of ethanol (mL)	1	2	3
Response (dependent variables)	Y_1: Particle size	$350 \leq Y1 \leq 500$ nm		
	Y_2: Entrapment efficiency (%)	$80 \leq Y2 \leq 100\%$		
	Y_3: Zeta potential	$-25 \leq Y3 \leq -35$mV		

Note: *X*1 and *X*2 is independent variables which affects the desirable responses (dependent variables) of *Y*1, *Y*2 and *Y*3.

2.4.5. Determination of AN by HPLC analysis

An HPLC instrument (Agilent Technologies Model-1260 infinity series, California) was used for the chromatographic separation using Zorbax Eclipse C_{18} RP column (150 × 4.6 mm; 5 μm, Agilent Technologies, California) at room temperature. Isocratic elution was carried out with mobile phase methanol and water (60: 40) at a flow rate 1 mL/min. Wavelength (λ) of UV detector was fixed at 225 nm.

AN (10 mg) was dissolved in 10 mL of methanol to make stock solution No.1. To make appropriate dilution of 0.5, 1.0, 1.5, 2.0 and 2.5 mL, solutions was taken from stock solution No.1 and diluted it up to 10 mL to receive concentration of 0.050, 0.100, 0.150, 0.200, 0.250 and 3.00 mg/mL respectively. Each 20 μl standard solution was introduced into the HPLC sample loop via a port on the injection valve and chromatogram was obtained. A calibration curve of standard AN was made to get peak area and retention time.

The stock solution treated as test sample solution of semi-purified AN extract was prepared in 10 mL of methanol by dissolving 10 mg semi-purified extract in it. The sample was sonicating up for 5 min to achieve complete dissolution, the solution allowed to equilibrate at room temperature. A total of 20 µl from the above samples was injected in six replicates. Relative standard deviation (RSD) values were calculated.

2.5. Formulation of nano-phytovesicular system

Preparation of nano-phytovesicular system (containing AN) was done according to the liquid dispersion technique with slight modification. The nano-phytovesicular system was composed of 1%–3% SPC (Phospholipon 90), 10%–30% ethanol, and 1% semi-purified AN extract of *A. paniculata* and water up to 100% as described by Sarwa, Mazumder, Rudrapal & Verma (2013). SPC and semi-purified AN extract was dissolved in ethanol then slowly added double distilled water in a fine streamlet with persistent merging at 700 rpm with a magnetic stirrer, (Remi India) in a well -closed container fabricated for preparation (fabricated in laboratory). Merging was continued for an extra 10–15 min. The system was kept at 30 °C throughout the preparation and then it was kept to cool down at room temperature. Finally, the obtained nano-phytovesicular system was kept in a closed container until estimation. A similar method was used for blank vesicular preparation without semi-purified extract.

2.6. Optimization of nano-phytovesicular formulation

Nano-phytovesicular system was otpimized using a 32 full factorial experimental design in order to evaluate the effect of formulation variables and experimental conditions using the Design-Expert 8 software (Stat-Ease, Inc., Minneapolis, MN). In experimental design, two factors were evaluated each at three levels and experimental trials with all nine possible combinations. The concentration of soya phasphatidylcholine (SPC) (X_1) and the volume of ethanol (X_2) were used as independent variables (Table 1). The vesicle droplet size (Y_1), percentage entrapment efficiency (Y_2) and zeta potential (Y_3) were stipulated as the dependent variables. An one-way analysis of variance (ANOVA) tests was executed to assess the level of significance of the tested factors of the selected responses (Sarwa, Das & Mazumder, 2014).

2.7. Characterization of nano-phytovesicular system

2.7.1. Vesicles morphology by TEM

Nano-phyto vesicles were visualized using a TEM (Hitachi, H-7500, Japan), with an accelerated voltage of 100 KV. One drop of each sample was placed on a microscopic carbon-coated copper grid to leave a thin film on the grids. Then, the film was negatively stained with 1% aqueous solution to phosphotungstic acid by placing a drop of the staining solution to the film and the excess of the solution was drained off with blotting filter paper. After drying, the specimen was viewed under the TEM at a 10–100 K fold enlargement (Sarwa et al., 2014).

2.7.2. Vesicles shape (morphology) by SEM

For scanning electron microscopy (SEM), one drop of nano-phyto vesicles system was mounted on a stub covered with clean glass. The drop was spread out on the glass homogeneously. A Jeol sputter coater (JEOL, Japan) was used to sputter-coat the samples of gold and the coated sample was examined under an SEM (JSM 6100, JEOL, Japan) at an accelerating voltage of 20 KV (Sarwa et al., 2014).

2.7.3. Vesicles size distribution by dynamic light scattering (DLS)

Size and size distribution of nano-phyto vesicles were determined by Brookhaven particle size analyzer with 90 plus particle sizing software Ver. 5.72 (Brookhaven Instrument Corp.) and Malvern zetasizer inspection system (MAL, 500962, Malvern, UK) without prior sonication. All measurement was done at 70 °C and at an angle of 90° between laser and detector. Before measuring, the samples were diluted appropriately with distilled water to prevent multiple scattering. The measurements were conducted in triplicate, in a multimodal mode at a medium count rate.

2.7.4. Zeta potential measurement

Zeta potential for the nano-phytovesicular preparations was determined by DLS using a computerized inspection system Malvern zetasizer (MAL, 5200962, Malvern, UK). Aliquots from preparation batches was sampled in disposable zeta cells distilled water was used as diluents for all samples and Zeta potentials were determined based on electrophoretic mobility under applied electrical field.

2.7.5. Entrapment efficiency

The amount of AN present in semi-purified extracts entrapped in nano-phytovesicular system of various formulations was determined by ultracentrifugation to separate the uninterrupted aliquot. The vesicular system was stored overnight at 4 °C in a deep freezer

(Remi, India) and then poured into 15 mL polycarbonate eppendorf tubes. The centrifugation procedure was carried out at 4 °C using centrifuge (Remi, India) equipped with a fixed angle rotator. The centrifugation speed was maintained at 1 5000 rpm for 2 h. The supernatant was removed and AN concentration before and after vesicular formulation was determined in HPLC analysis (Roy et al., 2010) in each case of calculation of entrapment efficiency (Sarwa et al., 2014). The entrapment efficiency (EE) (%) was calculated using following equitation:

$$EE = \mathrm{Qt} - \mathrm{Qs}/\mathrm{Qt} \times 100$$

Where EE is the entrapment efficiency, Qt is the amount of AN originally taken and Qs is the amount of AN detected in the supernatant.

2.7.6. Differential scanning calorimetry studies

Differential scanning calorimetry (DSC) analysis of nano-phytovesicular system was performed using a Perkin Elmer DSC instrument (Perkin Elmer Jade, USA). The transition temperature (Tm) was measured in aluminum crucibles of a heating rate 10 °C/min with 20 mL/min nitrogen gas flows within a temperature range of 40 to 300 °C. Sample weight around (10 ± 5) mg and temperature modulation amplitude from 0.01 to ±10 °C the peak transition onset temperatures of soya phosphatidylcholine, pure AN, semi-purified extract contain AN and nano-phytovesicular formulation were determined and compared.

2.7.7. Drug vesicle interaction study by FTIR

Lipid vesicle and semi-purified extract contain AN interactions were confirmed by fourier transform infrared (FTIR) study. The FTIR spectra of nano-phytovesicular system were recorded in the range of wave number 4000 to 400 cm^{-1} using Bruker automatic FTIR instrument (Bruke, Alfa-FTIR). The data stacked in under the Bruker instrument to compare and search for any chemical interactions.

2.8. In vitro absorption study

The modified drug absorption method was used in place of *in vitro* drug release study. *In vitro* method was used with slight modification to get large absorption profile of the formulation (Jain, Khurana, Pounikar, Gajbhiye & Kharya, 2013). *In vitro* experiment, two small parts (3 cm in length) of goat intestine were isolated, performed washing and cleaning to free from its unwanted intestinal contents. The intestine was averted and one corner of the intestine was tied tightly and another end was fitted with thread then it was kept in phosphate buffer saline (PBS) solution (pH=7.4). The free pure AN and nano-phytovesicular formulation (F-9) equimolar solutions containing AN were obtained by dissolving 4 mg of drug AN, and equivalent quantity of formulation (F-9) in 50 mL phosphate buffer saline were placed into six separate flask respectively. Ringer's solution (2.0 mL of mammalian) was injected into intestinal pieces then mixture were submerged individually in flasks containing free drug AN and nano-phytovesicular solutions. These flasks were stirred continuously at 37 °C for 2 h. The serosal fluid of each intestinal piece was withdrawn after the specified time period and assayed by HPLC for drug content by measuring concentration of drug absorbed. The cumulative absorption of AN was recorded and compared absorption of drug in free AN solution and nano-phytovesicular formulation.

2.9. In vivo *antihyperglycemic activity study*

2.9.1. Animals

Experimental male Albino Wistar rats weighing (200 ± 50) g was used in present experiment, under controlled temperature (24 ± 2) °C, 45%–50% relative humidity with natural day-night cycles (12: 12 h light and dark). All the animals were healthy and allowed to free access food and water given *ad libitum*. Prior approval was received from the IAEC (1576 /GO/a/11/CPCSEA), Department of Pharmaceutical Sciences, Dibrugarh University, Assam for this animal study protocol.

2.9.2. Acute toxicity

As per OECD (Organization for Economic Cooperation and Development) No. 425 guidelines, oral toxicity was performed. Experimental animals were provided pure AN and optimize nano-phytovesicular formulation (F-9) for next 3 h and animals were under observation for mortality and any other behavioral changes. Further next 48 h, animals were kept under observation for any mortality. Acute oral toxicity of prepared formulation of female Albino Wistar rat was determined as per reported method (OECD 425).

2.9.3. Oral glucose tolerance test (OGTT) of formulation

Overnight fasted normal animals were taken for OGTT study. Gum acacia solution (2%) and glibenclamide (GBL) 5 mg/kg were administered in six divided groups of rats. Pure AN (50 mg/kg), nano-phytovesicular formulation at a dose identical to 25 mg/kg AN and 50 mg/kg SPC, respectively. Body weight (2 g /kg) was faded when samples administered after half an hour. Sample blood was withdrawn by puncturing retro-orbital sinus at half an hour interval up to 120 min of sample administration. The blood sugar levels were evaluated by glucose oxidase-peroxidase reagent (Chattopadhyay, 1999).

2.9.4. Dosing and experimental procedures

Diabetes was induced by using diabetogenic agent streptozotocin (STZ). A solution of STZ (60 mg/kg body weight) was prepared in cooled citrate buffer in a pH of 4. 5. Prepared solution was introduced in intra peritoneal route with dose of 60 mg/kg body weight. Due to initial hypoglycemic induced by STZ, a 5% glucose solution was administered orally for 24 h to prevent mortality. After 48 h of SZT injection, a blood samples was drawn by retro-orbital sinus puncture. Blood glucose levels were tested in fasting condition and it exhibited in ranges of 300 to 320 mg/dL, which was suitable for study; glibenclamide (GLB) 5 mg/kg body weight was used as a reference drug (Babu, Gangadevi & Subramonium, 2003; Chattopadhyay, 1993). Initial toxicity study was planned and developed to demonstrate the adequate safe dose range of the AN, that could be used for subsequent experiment on the basis of previous study a fixed doses of AN was selected (Maiti, Mukherjee, Murugan, Saha & Mukherjee, 2010).

2.9.5. Protocol of experimental group

In present experiment rats were divided into six groups based on their normal plasma sugar levels and body mass. Group I was designated as vehicle control where normal rats received 0.5 mL of Tween 80 (5%), group II as diabetic control where diabetic rats given vehicle only. Group III was designated as standard drug group where diabetic rats were given GLB 5 mg/kg as oral hypoglycemic agent. Similarly group IV as treat group where diabetic rats received 50 mg/kg pure AN orally. Group V as treated group where diabetic rats were given nanophyto vesicle formulation and Group VI- as treated group where rats were given 50 mg/kg phospholipid with vehicle. Dose of AN in nanophyto formulation was equivalent to AN 25 mg/kg. The dose of drug was decided by available preliminary toxicity study.

2.10. Evaluation of antihyperglycemic activity

2.10.1. Bodyweight developments

Bodyweight of each animal was measured Weakley up to 21 d.

2.10.2. Biochemical estimation

After the estimation of blood sugar level, at the end of 21 d, the animals were sacrificed. Serum was separated out to determine the levels of serum triglyceride (TG), High density lipid (HDL) cholesterol, low density lipid cholesterol (LDL) cholesterol, total cholesterol (TC), blood urea nitrogen (BUN), insulin and creatinine levels using SPAN Diagnostics kit.

2.10.3. Histopathological studies

Pancreas and liver tissues were collected after sacrificing the rats by cervical dislocation and washed it in normal saline, fixed by using fixative for 24 h, and dehydrated with alcohol. After that cleaned it and embedded by using xylene and paraffin (m.p. = 58−60 °C). The tissues were stained by basic dye haematoxylin and the acid dye eosin to identify the nucleus and cytoplasm was used (Humanson, 1962; Jung et al., 2012).

2.11. Tissue distribution study

To assess the applicability of the work, the validated method was used to analyze different tissue samples after oral administration of *A. paniculata* extract at 133.33 mg/kg/d and AN at 100 mg/kg/d for four weeks. *In vivo* tissue distribution studies with AN was carried out in male Wister rats. The control group contained eight rats and treated with vehicle (0.5% w/v sodium-carboxymethyl cellulose solution in water containing 0.025% Tween 80) only. Forty-eight animals were divided into two groups (A and B) with 24 rats in each group. Furthermore, 24 rats of both the groups were divided into three sub-groups (A-I, A-II and A-III, $n = 8$; B-I, B-II and B-III, $n = 8$) for three different time points, *e.g.*, 1, 3 and 8 h, respectively. All animals of group A and B were administered AN and *A. paniculata* extract at 100 and 133.33 mg/kg/d for four weeks, respectively. The rats (150–200 g) were maintained for 28 d on 18% casein-containing semisynthetic diet with free access to food and water. On the 28th day, rats of all the groups and sub-groups were anaesthetized at predetermined time points (1, 3 and 8 h) and blood samples were collected by cardiac puncture. The animals were then sacrificed by cervical decapitation to collect different tissue samples. About 150 µL of the collected blood was centrifuged at 3500 rpm for 10 min at 10 °C for plasma and around 100 µL of the whole blood was stored for bioanalysis to determine the blood to plasma ratio. All tissues were rinsed immediately after collection with ice-cold saline and then stored at −80 °C.

In separate pharmacokinetic studies ($n = 4$), aminoguanidine (AG) was administered orally at 30 mg/kg to determine the pharmacokinetic parameters. Approximately 100 µL of blood was collected with EDTA-K_3 via retro orbital plexus at each time point (0, 0.17, 0.5, 1, 2, 3, 6, 8 and 10 h). The blood sample was centrifuged at 3500 rpm for 5 min at 15 °C to harvest plasma, which was stored at −20 °C until bioanalysis.

2.12. Statistical analysis

Obtained data were indicated as mean ± standard error of mean (SEM). For the antihyperglycemic activity, statistical analysis studies was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple test for comparison using Graph Pad Prism 5 (Graph-Pad Software, Inc., La Jolla, CA, USA). *P* values < 0.05 were considered significant according to method given in reference (Humanson, 1962; Jung et al., 2012).

3. Results

3.1. Identification and determination of AN in semi-purified extract

3.1.1. Determination of λ_{max}

The semi-purified extract from *Andrographis paniculata* contains AN and pure standard AN methanol solutions were scanned in UV–vis-Spectrophotometer the λ_{max} initiate to be 222 nm.

3.1.2. Determination of active compound by TLC

Thin layer chromatography (TLC) of semi-purified *A. paniculata* extract and pure AN was performed by using solvent system (chloroform:methanol = 7:1). The *Rf* value of pure AN was obtained and compared with semi-purified plant extract containing AN. The *Rf* value of the semi-purified compound was 0.61 and standard AN was 0.65, the TLC analysis was suggested that the extract having presence of AN.

3.1.3. Determination of AN by HPLC analysis

The semi-purified extract from plant *A. paniculata* containing AN was detected by HPLC analysis with explored RSD values of 0.373% in six replicates. Peak area in HPLC chromatogram and retention time was identified by comparison of sample solutions with the standard AN (Fig. 2). A linear plot was obtained and peak area (y) *versus* concentration (x) graph for AN was first prepared $y = 36601x+335.9$, $R^2=0.989$. Peak area in HPLC chromatogram and retention time (3.054 min) was used to detect AN concentration, purity of each peak was checked, and determined the concentration of AN present in semi-purified extracts. The HPLC analysis confirms that semi-purified extracts to contain AN 25.05 mg/gm semi-purified extract from plant *A. paniculata*.

3.2. Optimization of nano-phytovesicular formulation

Factorial design was selected to achieve proper planning and optimization of experimental series. In present experimental study, 32 factorial designs through Design-Expert8 software were used. The objective of optimization of pharmaceutical formulations is to determine the different levels of variables from which a strong product with high efficacy may be produced. Nano-phytovesicular formulations were optimized for the responses *Y*1 (PS), *Y*2 (EE), and *Y*3 (Zeta potential). The aim was to minimize particle size (PS) and poly despersity index (PDI) and maximize zeta potential and entrapment efficiency (EE). Therefore, for an optimized formulation facilitated for oral delivery of low absorption drug like AN, a reduced particle size, PDI, higher EE and good physical stability were prerequisites in order to improve the intestinal 10 absorption and oral bioavailability (Basalious, El-Sebaie & El-Gazayerly, 2011, 2010; Quinten et al., 2009). In experiment independent variables and the measured responses were shown in Table 2. Table 2 showed nine experimental runs (F1–F9) to accommodate two factors of three levels. The optimize formulation (F-9) constituents was selected based on dependent variables responses average particle size, EE% and Zeta-potential (de Lima, Araujo, Quinaia, Migliorine & Garcia, 2011). The predicted R^2 values were in a reasonable agreement with the adjusted R^2 in all responses and adequate precision and significant factor was shown in Table 3. The composition predicted and observed responses to the optimized formulation of nano-phytovesicles were presented in Table 4. The optimized nano-phytovesicular formulation (F-9) was composed of 100 mg semi-purifies AN extract, SPC 300 mg and 3 mL ethanol.

Results showed that the observed values of the optimized formulation were highly similar to the predicted values. On the basis of these results, it can be concluded that optimized nano-phytovesicular formulation provides a promising formulation of

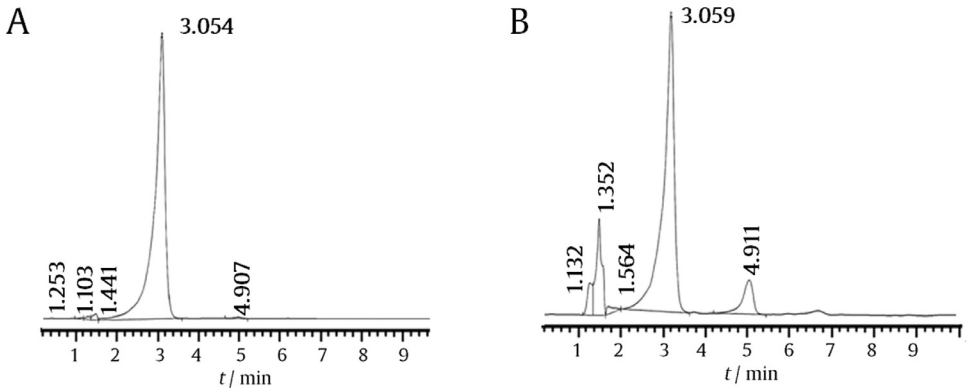

Fig. 2. HPLC chromatogram of standard AN (A) and semi-purified AN (B).

Table 2
Experimental runs, independent variables and measured responses of 3^2 full factorial experimental design.

Codes	SPC/mg	Ethanol/mL	Particle size/nm	Entrapment efficiency/%	Polydispersity index	Zeta potential/mV
F-1	100	2	402.0 ± 2.90	84	0.235 ± 0.015	−27.5
F-2	200	2	398.1 ± 2.04	82	0.245 ± 0.018	−28.8
F-3	300	2	405.2 ± 3.80	83	0.255 ± 0.015	−30.2
F-4	100	1	412.8 ± 5.70	82	0.253 ± 0.015	−26.5
F-5	200	1	422.0 ± 4.50	83	0.260 ± 0.240	−28.2
F-6	300	1	423.8 ± 1.80	86	0.336 ± 0.011	−28.4
F-7	100	3	398.0 ± 2.30	79	0.247 ± 0.029	−29.1
F-8	200	3	389.8 ± 11.00	75	0.251 ± 0.015	−31.5
F-9	300	3	395.5 ± 5.80	79	0.238 ± 0.008	−32.3

Note: F1 to F9 is formulation code prepared with different amount of formulation additives and its effects over response particle size, entrapment efficiency, polydispersity index and zeta potential.

Table 3
Output data of the 3^2 factorial analysis of all nano-phyto vesicular formulation.

Responses	R^2	R^2 Adjusted	R^2 Predicted	Adequate precision	Significant factors
PS	0.854	0.806	0.658	9.582	B
EE%	0.643	0.524	0.227	5.358	B
ZP	0.937	0.917	0.862	18.90	A & B

Note: The value of the R^2 indicates the precision in reproduction of the results and between predicted and actual response value in actual laboratory conditions.

Table 4
Composition of optimized formulation (F9) and its response on various physical parameters.

Variables	Repponses	Predicted values	Observed values
SPC Conc.	Particle size	394.58 nm	395.50 nm
Ethanol Volume	Entrapment efficiency	78.94%	79.00%
	Zeta potential	−32.10 mV	−32.30 mV

semi-purified plant extract containing AN therefore it was selected for further investigations.

3.3. Characterization of nano-phytovesicular system

3.3.1. Vesicles shape (morphology) by SEM and TEM

Vesicles shape micrographs of the nano-phytovesicular formulation by SEM was shown in Fig. 3A. The micrographs showed that the vesicles were clearly separated with spherical shape. The morphology and individual vesicles size of the optimized formulation was also observed under transmission electron microscopy (TEM) to get the internal matrix of vesicles. The TEM of the optimized formulation explored unilamellar vesicles in nano size range of with globular shapes. Fig. 3B clearly indicates that the size range of the vesicles observed in the micrographs was in accordance with the data obtained by particle size analysis.

3.3.2. Vesiclessize distribution by DLS

Vesicle size and size distribution of nano-phytovesicles formulation containing AN were in nano size range (389.8 ± 11.00) to (422.0 ± 4.50) nm. The optimized nano-phytovesicles formulation (F-9) determined average vesicle size of (395.5 ± 5.80) nm (Table 2).

3.3.3. Zeta potential measurement

This study is useful as it provides information about the surface charge of the vesicular system. The Zeta potential plays a crucial role in determining the stability of the prepared formulation. Malvern zetasizer was used to determine surface charge of the nano-phytovesicles formulation. The optimize formulation (F-9) having Zeta potential of −32.3 mV stabilized the vesicles separately for long time (Table 2).

3.3.4. Entrapment efficiency

All EE estimations were carried out in a reverse phase HPLC system. The EE plays important role in drug delivery and absorption. The higher drug entrapment shows the better drug delivery and higher absorption. In present experiment the nano-phytovesicualr systems having the less particle size with high EE (79%) of AN were clearly shown in the optimize formulation (F-9) (Table 2).

3.3.5. DSC studies

DSC was used to investigate the nature of the drug (crystalline or amorphous) in a prepared formulations and find out possibilities of interactions with other mixed ingredients. Fig. 4A, C and E showed the DSC thermograms of semi-purified AN extract, SPC, and optimized nano-phytovesicular preparations commonly. The

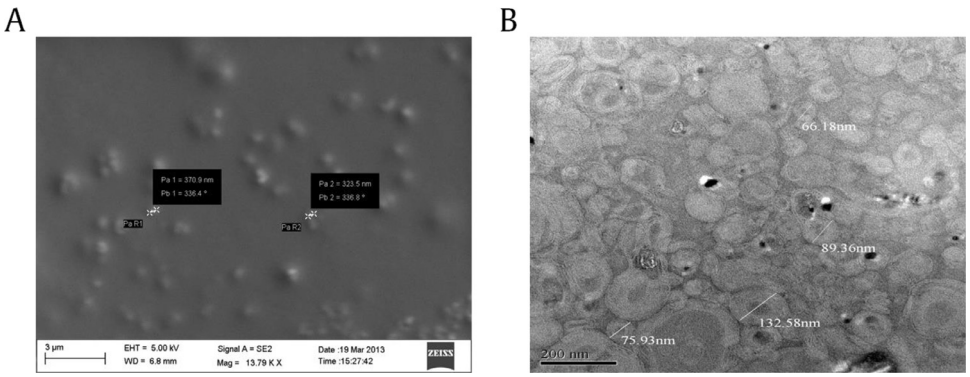

Fig. 3. SEM (A) and TEM (B) micrographs of nano-phyto vesicular optimized formulation.

DSC scan of large amount of SPC explored two broad endothermic peaks which were characteristics of amorphous substances at 167.43 °C, and 165.24 °C showed the lipid movements in hot condition can change gel to crystalline state. The prepared nano phytovesicular system consisted of SPC lipid, while semi-purified extracts was present in the lipid bilayer. In the DSC, thermogram a sharp peak of a maximum at 126.89 °C appeared in the semi-purified AN extract (Fig. 4A), but no other endothermic or exothermic peaks were observed. In formulation, (Fig. 4C) a depression of the melting point of plant extracts containing AN and SPC from 126.89 °C and three new peaks with maxima at 113.59 °C, 139.12 °C and 153.69 °C respectively appeared in the nano-phytovesicular formulation. At the same time, the melting enthalpy values of AN and SPC showed drastic depression from 175.25 J/g and 57.70 J/g to 164.79 and 1.219 J /g, respectively, compared to their bulk materials (Hou, Xie, Huang, & Zhu, 2003). The overall interactions of SPC and semi-purified AN extract nano-phytovesicular formulation were concluded by elimination of endothermic peak (s), appearance of new peak (s), change into peak shapes and its onset, peak temperature/melting point and relative peak area or melting enthalpy shown in Fig. 4E.

3.3.6. Drug vesicle interaction (FTIR) study

FTIR is a fast and novel technique which gives suitable chemical data onto peak combination and supportive subtraction. To examine the drug-excipient interactions the FTIR analysis was performed before formulation development. Drug-excipient interaction studies plays vital role during preformulation study. It have explored much information about the stability of formulations and drug availability patterns (Mukherjee, Mahapatra, Das & Patra, 2005). The medium infrared range consists 4000–400 cm^{-1} is an very useful area of analysis, providing qualitative information on functional groups as well as offering the opportunity for quantitative analysis (Hakuli, Kytokivi, Lakomaa & Krause, 1995). FTIR spectrometric analysis of soy lecithin showed peak at 3570 cm^{-1} and 3313cm^{-1}, which denoted the presence of N-H and O-H stretching of amino group and hydroxyl group respectively. Another band of 2943–2831 cm^{-1} indicated the presence of C-H group of alkane. Band of 1448.21 cm^{-1} presented the ester linkage. Another band at 1571.82 cm^{-1} was found due to aliphatic alkane. Band at 1245 cm^{-1} indicated presence of P=O (phosphomoyl) group.

Fig. 4B, D and F showed the FTIR spectrum of semi-purified AN extract, SPC, and nano-phytovesicles formulation. FTIR spectrum of nano-phyto vesicles formulation showed the minor shifting of some peaks compared to semi-purified extract and SPC excipient, like aliphatic alcoholic O-H stretches (3396.13–3281.23 cm^{-1}), phenolic C-O stretch (1326.028– 1418.07 cm^{-1}), and C-O stretch of ether (1043.33–1085.27 cm^{-1}) (Zhao & Feng, 2005). The FTIR spectra of semi-purified extract (AN) phyto-SPC vesicular formulation was found to be significantly different from SPC and semi-purified AN extract that support the reaction of –OH group of semi-purified AN at choline part of SPC, which indicated the formation of phytolipid vesicular system.

3.4. In vitro *absorption study*

In *in vitro* absorption, study showed higher absorption of semi-purified AN extract from nano-phytovesicles than plain AN solution. The enhanced absorption and maximum cumulative release of AN from the nano-phytovesicular formulation recorded at different time intervals as compared to the AN alone clearly indicated in Fig. 5.

3.5. In vivo *antihyperglycemic activity study*

3.5.1. Acute toxicity

The rats mortality was not recorded at the dose of pure AN 500 mg/kg yet. Hence, based on previous studies and tenth parts of tested dose, *i.e.* 50 mg /kg body weight was selected for the study. The nano-phytovesicular formulation containing AN a dose of 25 mg/kg was selected for the study.

3.5.2. Developments in bodyweight

The alteration of blood glucose level impacts on body weight in STZ-induced hyperglycemic rats was found significant ($P < 0.01$) when compared it to control group from day 1, 7, 14 and 21 d (Table 5). Regular daily application of pure AN and nano-phytovesicular formulation supplement at 25 mg/kg body weight to diabetic group animal gave significantly improved body weight. Mostly increase to body weight detected with nano-phyto formulation and in standard drug GBL at 5 mg/kg body weight dosing group, no significance changes were observed as compared to diabetic group.

3.5.3. Oral glucose tolerance test

Glucose level in a blood was decreased in both pure AN and AN extract loaded nanophyto vesicle formulation, the extent of effect was differ in different time interval reported in Table 6. The nanophyto vesicle formulation loaded with AN showed significant effect than pure AN ($P < 0.01$).

3.5.4. Variations in blood glucose level

During fasting glucose on the STZ-induced hyperglycemic rats in consecutive days of experiment starting from day 1, 7, 14 and 21, the rats showed significant ($P < 0.01$) increase as compared to the base values. Oral use of standard drug GLB 5 mg/kg,

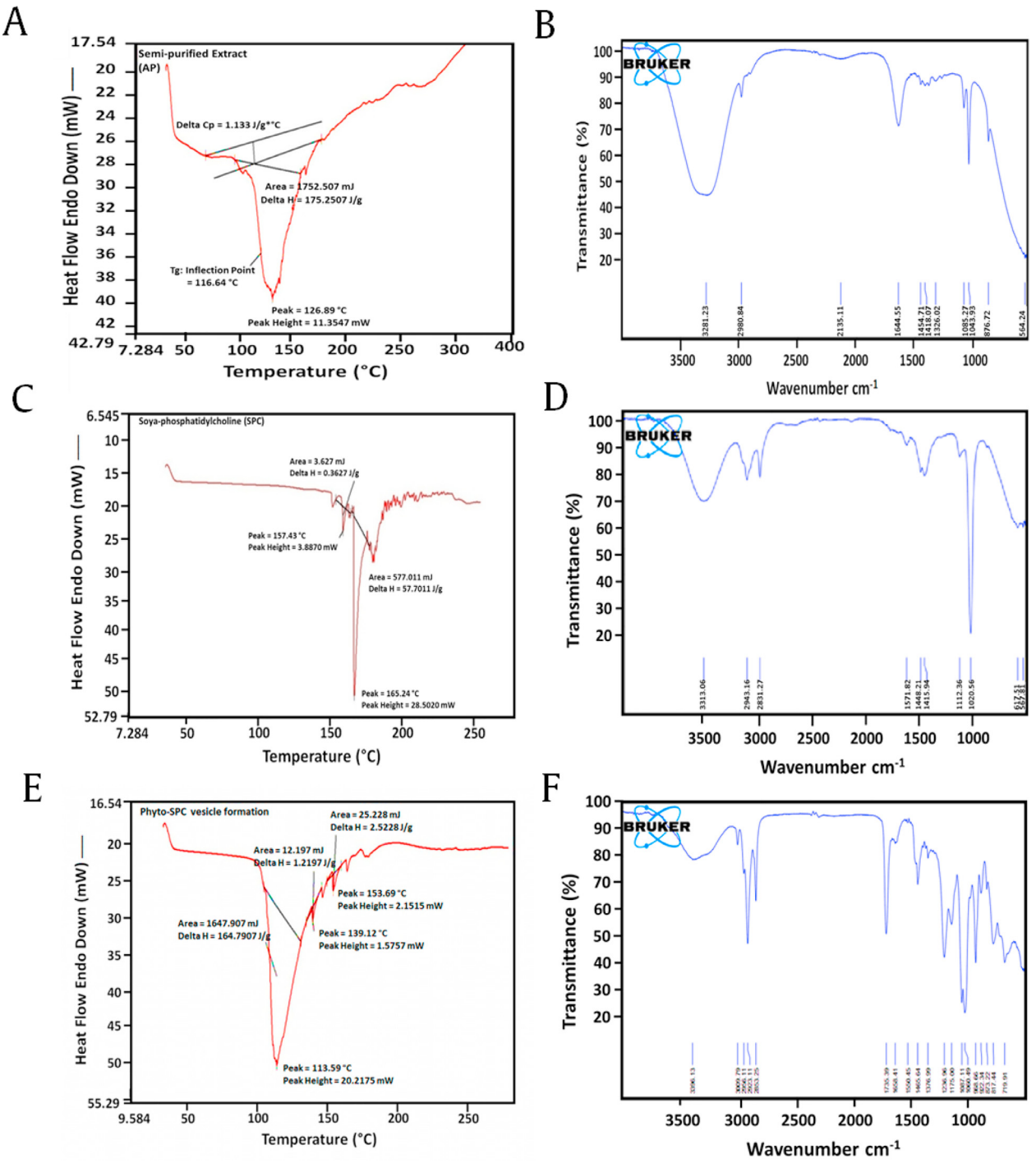

Fig. 4. DSC thermogram (A–E) and FTIR spectrum (B–F) of semi-purified AN extract, SPC and nano-phyto vesicular formulation, respectively.

Table 5
Effects of nano-phyto vesicular formulation (F9) on body weight changes in rats (mean± SEM, $n = 6$).

Animal groups	1 days	7 days	14 days	21 days
G-I Vehicle control	193.3 ± 3.29	212.8 ± 3.18##	231.0 ± 2.06##	239.3 ± 1.54##
G-II STZ diabetic control	193.3 ± 3.16	186.3 ± 1.89**	163.6 ± 2.61**	148.6 ± 2.52**
G-III Std. drug (GLB)	191.16 ± 2.52	197.3 ± 1.35**#	199.1 ± 3.34**##	192.6 ± 2.23**##
G-IV Pure-AN (50 mg/kg)	189.0 ± 3.40	193.5 ± 2.81**	192.6 ± 2.23**##	174.6 ± 2.15**##
G-V Nano-phyto vesicle (25 mg/kg)	189.83 ± 3.21	198.5 ± 2.46**##□	195.0 ± 2.89**##□□	190.8 ± 3.75**##□□
G-VI SPC (50 mg/kg)	191.5 ± 2.47	188.3 ± 2.60**	165.0 ± 2.47**	156.0 ± 2.47**

* $P < 0.05$ **$P < 0.01$ *vs* vehicle control group I.
\# $P < 0.05$ \#\#$P < 0.01$ *vs* STZ diabetic control group II.
□ $P < 0.05$ □□ $P < 0.01$ compared in between group VI and group IV. Data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple test for comparison.

Table 6
Effect of nano-phyto vesicular formulation (F9) on blood glucose level (mg/dL) of rats tested by OGTT (mean± SEM, $n = 6$).

Animal groups	0 min	30 min	60 min	90 min	120 min
G-I Vehicle Control	76.6 ± 1.70	166.0 ± 4.73#	138.5 ± 1.83##	124.3 ± 1.83##	112.5 ± 2.55##
G-II Std. Drug (GLB)	79.6 ± 2.51	151.5 ± 3.27*	127.6 ± 2.63**	105.3 ± 1.92**	87.66 ± 2.06**
G-III Pure -AN (50 mg/kg)	84.8 ± 7.07	145.6 ± 2.17**	137.33 ± 3.79#	123.5 ± 3.09##	105.0 ± 3.75*##
G-IV Nano-phyto vesicle (25 mg/kg)	76.6 ± 2.38	136.3 ± 1.94**#	127.8 ± 2.68**	110.0 ± 2.74**	89.5 ± 2.65**
G-V SPC (50 mg/kg)	76.1 ± 2.32	149.6 ± 3.37**	140.3 ± 2.01##	124.0 ± 2.50##	114.6 ± 2.72##

* $P < 0.05$ **$P < 0.01$ vs vehicle group I.
# $P < 0.05$ ##$P < 0.01$ *vs* standard drug (GLB) group II. Data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple test for comparison.

Table 7
Effect of nano-phyto vesicular formulation (F9) over Blood glucose level (mg/dl) of STZ-induced diabetic rats (mean± SEM, $n = 6$).

Animal groups	1 days	7 days	14 days	21 days
G-I Vehicle Control	78.6 ± 3.07##	77.3 ± 2.41##	76.6 ± 2.36##	71.3 ± 2.61##
G-II STZ Diabetic Control	309.5 ± 2.77**	297.8 ± 2.91**	311.3 ± 3.08**##	283.3 ± 4.46**
G-III Std. Drug (GLB)	311.3 ± 2.81**	230.8 ± 3.63**##	128.5 ± 3.56**##	86.0 ± 4.69*##
G-IV Pure- AN (50 mg/kg)	313.0 ± 3.10**	237.1 ± 2.96**##	168.5 ± 4.00**##	102.0 ± 4.01**##
G-V Nano-phyto vesicle (25 mg/kg)	309.1 ± 3.19**¤	217.0 ± 2.68**##¤	131.1 ± 3.53**##¤	87.3 ± 3.32*##¤
G-VI SPC (50 mg/kg)	316.8 ± 4.46**	303.5 ± 3.75**	287.8 ± 3.49**##	281.5 ± 3.47**

* $P < 0.05$ **$P < 0.01$ *vs* vehicle group I.
# $P < 0.05$ ##$P < 0.01$ *vs* STZ diabetic control group II.
¤ $P < 0.05$ ¤¤$P < 0.01$ compared in between group VI and group IV. Data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple test for comparison.

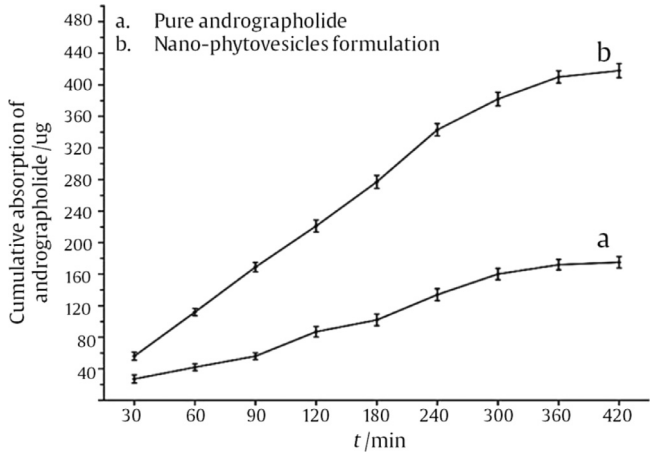

Fig. 5. Cumulative absorption *versus* time graph of pure AN and nano-phyto-vesicular formulation containing semi-purified AN.

pure AN 50 mg/kg and nano-phytovesicular formulation equivalent 25 mg/kg AN amount formed remarkable ($P < 0.01$) decrease in blood glucose mainly nano-phytovesicular formulation on consecutive experimental days as related to control of vehicle as well as diabetic control group. Group V nano-phytovesicular formulation treated rats showed significant reduction in blood glucose levels when compared with group IV pure AN treated rat (Table 7).

3.5.5. Variations in biomarkers

LDL-cholesterol, total cholesterol (TC) and TG showed elevation in STZ-induced hyperglycemic rats serum content, whereas HDL-cholesterol was significantly ($P < 0.01$) decreased as compared to the control of vehicle group. Everyday oral dealing of standard drug GLB 5 mg/kg and nano-phytovesicular formulation equivalent to AN 25 mg/kg body weight dose presented important ($P<0.01$) decrease of LDL, TC and TG and instantaneously rise the HDL levels as comparing to the control of diabetic group. Group V nano-phytovesicular formulation treated rats showed significant changes in HDL, LDL, TC and TG levels when compared with group IV pure AN treated rats (Table 8). The blood urea nitrogen and creatinine level higher although the insulin level in STZ-induced hyperglycemic rats fell as compared to control group. On treatment of nano-phytovesicular formulation, significantly ($P < 0.01$) restore the renal function marker levels as compared to control of vehicle as well as control of diabetic group. Group V nano-phytovesicular formulation treated rats showed significant changes in renal functional marker blood urea nitrogen, creatinine and insulin levels when compared with group IV pure AN treated rats (Table 9).

3.6. Histopathological study

On treatment of nano-phytovesicular formulation (F-9) showed dramatic restore of abnormal histological changes in STZ-induced hyperglycemic rats like degeneration and necrosis of the islets in the pancreas, reduction in numbers, size, vacuolation and atrophy of pancreatic islets as well as its connective tissue incursion in the parenchyma and compare with other treated groups (Fig. 6). The cytoplasm of peri-acinar hepatocytes showed either a single large or multiple small round empty vacuoles that distended the cell cytoplasm 15 and displaced the nucleus to the periphery in histological sections of liver stained with haematoxylin and eosin. These degenerative changes were also seen, to a lesser extent, in the mid-zonal regions. Parenchymatous degeneration was observed in the peripheral regions. Dissociation of hepatocytes and sinusoidal dilatation occurred due to these changes (Fig. 7).

Pharmacokinetic studies showed that AN gets quickly absorbed and extensively metabolised in rat and human (Panossian et al., 2000), isolated ten sulfonated AN metabolites from rat urine, feces and the content of the small intestine after the drug was orally administered to rat (He et al., 2003a, 2003b). Among them, one metabolite, 14-deoxy-12(*R*)-sulfo AN, was found to be identical to an injectable anti-inflammatory drug (Lianbizhi) currently in the clinical use in China (Cui, Qiu, Wang & Yao, 2004; Meng, 1981) identified 12 new AN metabolites in human urine after oral administration, three of which were characterized as 3-*O*-sulfate conjugates, one as a 3-*O*-sulfate-12-*S*-cysteine conjugate and the remaining eight as 19-*O*-β-D-glucuronide (Cui et al., 2004 Cui, Qiu

Table 8
Effect of nano-phyto vesicular formulation (F9) over serum lipid profile in STZ-induced diabetic rats (mean± SEM, $n = 6$).

Animal groups	HDL/(mg•dL^{-1})	LDL/(mg•dL^{-1})	Total cholesterol/(mg•dL^{-1})	TG/(mg•dL^{-1})
G-I Vehicle Control	37.33 ± 2.41##	38.67 ± 3.55##	81.00 ± 3.18##	75.50 ± 3.50##
G-II STZ Diabetic Control	16.50 ± 1.70**	90.83 ± 2.42**	164.80 ± 3.28**	161.50 ± 3.46**
G-III Std. Drug (GLB)	29.50 ± 1.78*##	39.33 ± 3.15##	99.83 ± 3.71##	78.33 ± 3.53*##
G-IV Pure- AN (50 mg/kg)	26.83 ± 1.62**##	53.83 ± 3.95*##	122.30 ± 5.20##	84.50 ± 4.95**##
G-V Nano-phyto vesicle (25 mg/kg)	30.83 ± 2.92##¤	38.33 ± 3.40 ##¤¤	92.17 ± 4.54 ##¤¤	74.67 ± 4.55##¤
G-VI SPC (50 mg/kg)	17.83 ± 1.57**	76.00 ± 4.52**#	153.70 ± 5.38**	147.70 ± 3.25**

* $P < 0.05$ ** $P < 0.01$ *vs* vehicle group I.
# $P < 0.05$ ## $P < 0.01$ *vs* STZ diabetic control group II.
¤ $P < 0.05$ ¤¤ $P < 0.01$ compared in between group VI and group IV. Data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple test for comparison.

Table 9
Effect of nano-phyto vesicular formulation (F9) over Serum biochemical parameters in STZ-induced diabetic rats (mean± SEM, $n = 6$).

Animal groups	BUN/(mg•dL^{-1})	Insulin/(ng•mL^{-1})	Creatinine/(mg•dL^{-1})
G-I Vehicle Control	30.44 ± 0.54##	0.53 ± 0.022##	0.48 ± 0.014##
G-II STZ Diabetic Control	204.50 ± 1.36**	0.26 ± 0.016**	4.22 ± 0.148**
G-III Std. Drug (GLB)	121.70 ± 1.17**##	0.46 ± 0.026##	2.38 ± 0.151**##
G-IV Pure- AN (50 mg/kg)	151.20 ± 2.61**##	0.34 ± 0.018**#	3.52 ± 0.178**##
G-V Nano-phyto vesicle (25 mg/kg)	123.60 ± 2.66**##¤	0.43 ± 0.020*##¤	2.95 ± 0.076**##¤¤
G-VI SPC (50 mg/kg)	184.00 ± 3.30**##	0.28 ± 0.026**	4.11 ± 0.157**

* $P < 0.05$ ** $P < 0.01$ *vs* vehicle group I.
# $P < 0.05$ ## $P < 0.01$ *vs* STZ diabetic control group II.
¤ $P < 0.05$ ¤¤ $P < 0.01$ compared in between group VI and group IV. Data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple test for comparison.

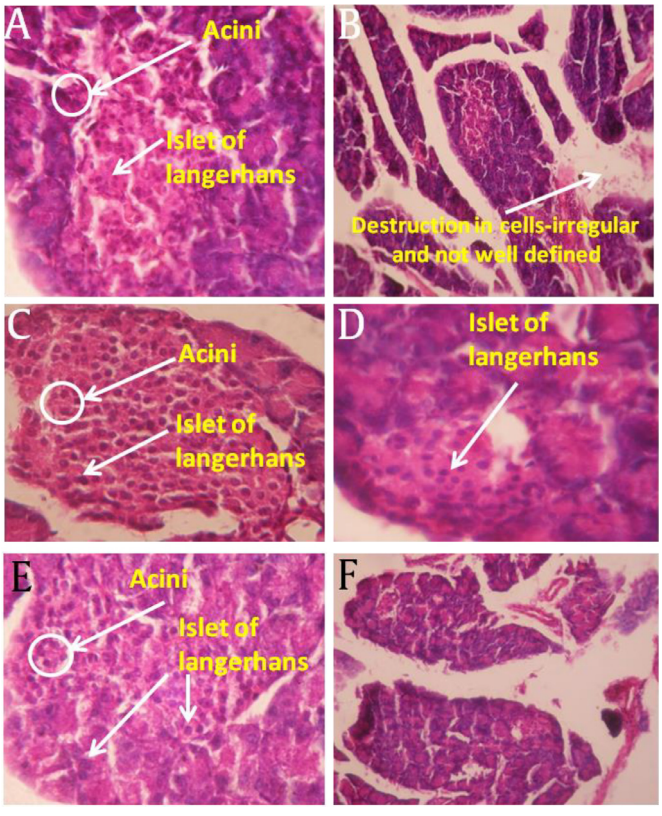

Fig. 6. Histological examinations of 21 d experimental rat pancreas. *Note:* A: Pancreatic sections of normal rat showing cells with well-preserved cytoplasm and nucleus; B: STZ induced toxic rats pancreatic sections, the cells were irregular, not well defined and defect in cell membrane. Necrosis of the cells was very clear; C: Standard drug GLB treated group; D: Free pure AN treated group; E: Nano-phyto vesicular formulation treated rats restored the altered histopathological changes; F: SPC treated group.

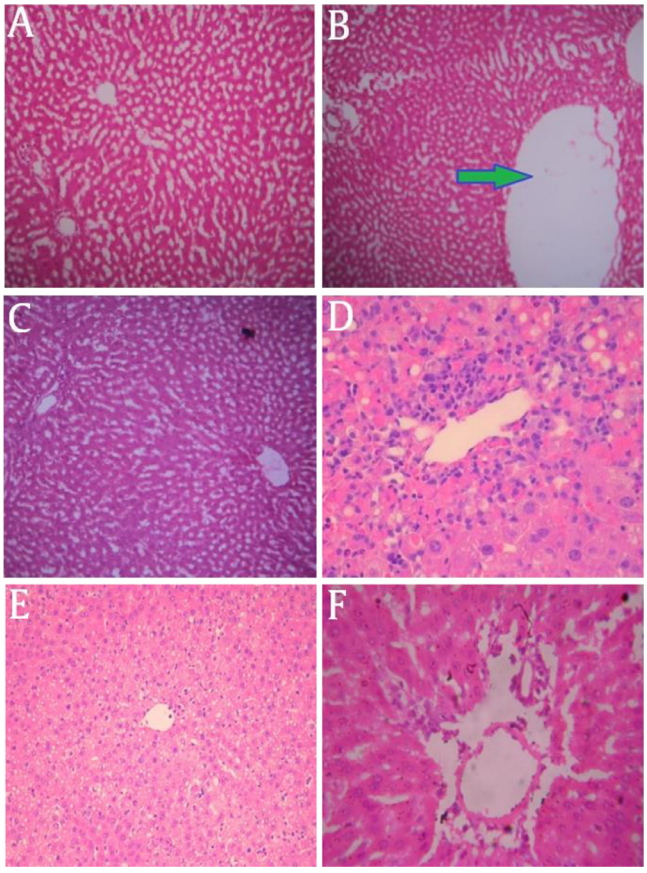

Fig. 7. Liver histopathological examinations of 21 d experimental rats. Note: A: vehicles control, the rats showing normal histological sections, well arranged cells and clear central veins. B: STZ-induced diabetic controlled rat group; C: Standard drug GLB treated group (all figures were in 10 × magnification); D: Free pure AN treated group; E: nano-phyto vesicular formulation treated rats restored near to normal rats liver histology; F: SPC treated rat group.

& Yao, 2005). Cui et al. also demonstrated that AN was extensively metabolized through variety of pathways to form a large number of metabolites in humans. The pathways include oxidation, hydrolysis, isomerization, glucuronidation, sulfation and the conjugation of urea, cysteine, creatinine and hydrazine (Cui et al., 2005). Zhao et al. studied comparative in-vitro metabolic profiles of AN using pooled human (HLMs), dog (DLMs) and rat (RLMs) liver microsomes. Eight phase I (M1-1 to M1-8) and five phase II (M2-1 to M2-5) metabolites resulted from dehydration, deoxygenation, hydrogenation and glucuronidation were identified by accurate mass measurement and MS/MS fragmentation RSC Advances (Zhao et al., 2002).

4. Discussion

Results elaborated about semi-purified AN extract as nano-phytovesicular carrier for enhancing oral absorption and hypoglycemic activity with adequate extent of data and evidence. Herbal drugs contain various compounds and the efficacy of these herbal drugs mainly depends on the major active ingredient delivering an effective level to the target site or in blood plasma for long time. AN is a well-known chemical constituent of *A. paniculata*used in Indian traditional schemes as well as China for dealing number of disorders. Although it's well-known hepatoprotective and antihyperglycemic action, AN takes limited bioavailability. Though it is poor oral absorption, its quick clearance drops its elimination half-life, enhances the plasma protein binding of AN and reduces its bioavailability (Bombardelli, 1994; Maiti et al., 2006; Rajagopal et al., 2003; Sharma, Gupta & Dixit, 2010).

These problems necessitate developing some novel herbal dosage form as in conservative form that may manage the AN concentration in blood for a long time period. The lipid vesicles system contains phospholipids play a major role in drug delivery technology as carrier system due to their property to improve oral absorption of plant constituents of complex formation (Yanyu, Yunmei, Zhipeng & Qinenq, 2006; Yu et al., 2003). The semi-purified AN extract from plant were characterized and identified by UV–vis-Spectrophotometer, TLC, FTIR, DSC, and HPLC analysis, the concentration of AN in the semi-purified extract determined through HPLC analysis was found to be 25.054 mg/gm of semi-purified extract. In the present study, an attempt was made to formulate a novel dosage form of semi-purified AN extract from *A. paniculata* to overcome these shortcomings. Nano-phytovesicular formulation containing semi-purified AN extract was made by a simple and reproducible technique. The formulation of nano-phytovesicular system was optimized with the help of independent and dependent variables responses used in formulation process using the Design Expert 8 software. The optimized formulation (F-9) having the high drug entrapment as well as the minimum vesicle size of optimal stability which is the basic requirement of the vesicular carrier system. The physical characterization showed vesicular system having nano sized range with high drug EE and good stability (Yu et al., 2003).

The nano-phytovesicular system produced antihyperglycemic activity for a long period of time and normalized at adverse situations of rat pancreas and liver additional competently as compare to drug free condition. The outcomes reveals that nano-phytovesicular system of the semi-purified extract is equivalent to 25 mg/kg AN has better effect than produced to 50 mg/kg free AN, the better effect is due to the long term retention of the formulation containing AN. The nano-phyto vesicles having protective effect on the hyperglycemias may be due to AN containing vesicular formulation of semi-purified extract from plant combined with SPC and form complex thus sustained the release property. Complexation shows important role in nourishing AN relief from the nano-phyto vesicles, which is marked from the experimental outcomes. Therefore, the developed sustained release design can be useful in lower the quantity and frequency of ordered dosage.

The *in vitro* drug absorption of nano-phyto vesicles through intestine was compared with plain AN. The averted intestine sac method was used to study has an advantage over the conventional dissolution study with correlation between absorption technique. Comparatively small amount of serosal fluid can measure large changes. In averted intestine sac method, the epithelial cells of the mucosal surface exposed directly to the oxygenated mucosal fluid (Barthe, Woodley, Kenworthy & Houin, 1998). The mucosal fluid absorbing drug from the serosal fluid through the intestine epithelial cells at various time intervals was observed, which showed significantly higher level of AN from the nano-phyto vesicles when compared with equimolar doses of plain AN solution. Due to the amphiphilic nature of the nano vesicles, water and lipid solubility as well as the oral absorption of the AN has greatly enhanced.

In vivo antihyperglycemic study showed that the nano-phytovesicles of semi-purified AN extract was the most effective against all the treated groups in terms of lowering the blood glucose level and enhancing in body weight. There was being increased in total cholesterol, triglycerides and LDL cholesterol associated with decrease in HDL cholesterol in diabetic control group animals. On treatment of nano-phytovesicular formulation, the total cholesterol, triglycerides and LDL cholesterol was reduced in 21 d treatment as well as the HDL cholesterol level was significantly increased. Simultaneously, the increased blood urea nitrogen levels and serum creatinine increase in diabetic control group animals that are significant marker of renal insufficiency associated to coronary artery disease was found to be reduced on treatment. Insulin deficiency in β–cells was increased on treatment from nano-phytovesicles containing AN and improved on pancreatic exocrine activities may be due to enhance level of secretion of insulin from remaining residual β–cells of islets or because of enhancing passage of blood glucose to peripheral (Shen et al., 2002).

The histopathological studies of pancreas and liver indicate that nano-phytovesicle treated groups able to improve the cytoprotective action of extract. The photomicrograph reports displayed that the insulin production from β–cells is situated in central regions of pancreatic islets, and reserve of these histomorphological variations to maintain the normal construction of producer cells of insulin via AN nano-phytovesicular formulation create to shield from the STZ- induced damage of above cells.

Study shows the AN's absolute oral bioavailability indicated that this compound has very poor oral bioavailability (only 2.67%). Biological stability study indicated that AN was rapidly metabolized in the duodenum and jejunum, forming a sulfate that is hydrophilic and likely to be impermeable. Mechanistic disposition studies indicated that poor absorption was not the reason for its poor bioavailability at low dose, although it may contribute to its poor bioavailability at high dose because absorption from terminal ileum and colon was much slower. The poor absorption from terminal ileum and colon was due to the presence of efflux transporter(s) that can be overcome by a combination of P-gp and Bcrp inhibitors. Taken together, the rapid sulfation of AP is likely the main cause of AP's low bioavailability, whereas efflux at the terminal ileum and colon contributes to AP's low bioavailability at high doses.

5. Conclusion

The present work concludes that novel herbal formulation improves pharmaceutical properties, antihyperglycemic activity and enhances the efficacy of herbal drugs. The semi- purified AN extract from *A. paniculata* plants was converted in to novel nano-phytovesicles delivery system and increased antihyperglycemic activity. Enhancement of activity and reduction of dosage due to in-

creased oral absorption and longtime retention of semi-purified AN extract in blood plasma.

Declaration of Competing Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgements

Dr. Vinod Kumar Verma is grateful to University Grant Commission, Government of India, New Delhi for financial support through UGC-JRF in Engineering and Technology (Vide-F No. 10-01/2008 SA-I). The authors greatly acknowledge with thanks Late Prof. (Dr.) Ashoke Kumar Dolui for his novel ideas, righteous contribution and invaluable guidance toward the work presented. We extend our sincere thanks to Ludwigshafen GmbH for providing Lipoid. The authors also acknowledge Department of Pharmaceutical Sciences, Dibrugarh University Assam for providing the institutional and instrumental facilities.

References

Ahmad, M. S., Ahmad, S., Arshad, M., & Afzal, M. (2014). *Andrographia paniculata* a miracle herbs for cancer treatment: *In vivo* and *in vitro* studies against aflatoxin B1 toxicity. *Egyptian Journal of Medical Human Genetics, 15*, 163–171.

Akbarsha, M. A., & Murugaian, P. (2000). Aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats: Effect on the testis and the cauda epididymidal spermatozoa. *Phytotherapy Research, 14*, 432–435.

Arien, A., & Dupuy, B. (1997). Encapsulation of calcitonin in liposomes depends in the vesicle preparation method. *Journal of Microencapsulation, 14*, 753–760.

Arien, A., Goigoux, C., Baquey, C., & Dupuy, B. (1993). Study of *in vitro* and *in vivo* stability of liposomes loaded with calcitonin or indium in the gastrointestinal tract. *Life Sciences, 53*, 1279–1290.

Babu, V., Gangadevi, T., & Subramonium, A. (2003). Antidiabetic activity of ethanol extract of *cassia kleinii* leaf in streptozotocine-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian Journal of Pharmacology, 35*, 290–296.

Barthe, L., Woodley, J. F., Kenworthy, S., & Houin, G. (1998). An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine. *European Journal of Drug Metabolism and Pharmacokinetics, 3*, 313–323.

Basalious, E. B., El-Sebaie, W., & El-Gazayerly, O. (2011). Application of pharmaceutical qbd for enhancement of the solubility and dissolution of a class ii bcs drug using polymeric surfactants and crystallization inhibitors: Development of controlled-release tablets. *AAPS PharmSciTech, 12*, 799–810.

Basalious, E. B., Shawky, N., & Badr-Eldin, S. M. (2010). SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: Development and optimization. *International Journal of Pharmaceutics, 391*, 203–211.

Bhan, M. K., Dhar, A. K., Khan, S., Lattoo, S. K., Gupta, K. K., & Choudhary, D. K. (2006). Screening and optimization of *andrographis paniculata* (Burm. f.) nees for total andrographolide content, yield and its components. *Scientia Horticulturae, 107*, 386–391.

Bombardelli, E. (1994). Phytosomes in functional cosmetics. *Fitoterapia, 65*, 320–327.

Bothiraja, C., Pawar, A. P., Shaikh, K. S., & Sher, P. (2009b). Eudragit epo based nanoparticles suspension of andrographolide: *In vitro* and *in vivo*. *Nanoscience and Nanotechnology Letters, 10*, 156–164.

Bothiraja, C., Shinde, M. B., Rajalakshmi, S., & Pawar, A. P. (2009a). Evaluation of molecular pharmaceutical and *in vivo* properties of spray-dried isolated andrographolide-PVP. *Journal of Pharmacy and Pharmacology, 61*, 1465–1472.

Brahmachari, G. (2017). *Andrographolide: A molecule of antidiabetic promise, natural product drug discovery* (pp. 1–27). Elsevier Science.

Calabrese, C., Berman, S., Babish, J., Ma, X., Shinto, L., Dorr, M., et al. (2000). Aphase i trial of andrographolide in hiv positive patients and normal volunteers. *Phytotherapy Research, 14*, 333–338.

Chadha, Y. R. (1985). In *The wealth of India: Raw materials: Vol. 1 A* (pp. 264–270). New Delhi, India: Council of Scientific and Industrial Research.

Chattopadhyay, R. R. (1993). Hypoglycemic effect of *ocimum sanctum* leaf extract innormal and streptozotocin diabetic rats. *Indian Journal of Experimental Biology, 31*, 891–893.

Chattopadhyay, R. R. (1999). A comparative evaluation of some blood sugar lowering agents of plant origin. *Journal of Ethanopharmacology, 67*, 367–372.

Chinsembu, K. C. (2019). Diabetes mellitus and nature's pharmacy of putative antidiabetic plants. *Journal of Herbal Medicine, 15*, 100–230.

Cui, L., Qiu, F., Wang, N., & Yao, X. (2004). Four new andrographolide metabolites in human urine. *Chemical and Pharmaceutical Bulletin, 52*, 772–775.

Cui, L., Qiu, F., & Yao, X. (2005). Isolation and identification of seven glucuronide conjugates of andrographolide in human urine. *Drug Metabolism Disposition, 33*, 555–562.

de Lima, L. S., Araujo, M. D. M., Quinaia, S. P., Migliorine, D. W., & Garcia, J. R. (2011). Adsorption modeling of Cr, Cd and Cu on activated carbon of different origins by using fractional factorial design. *Chemical Engineering Journal, 166*, 881–889.

Gupta, A., Ashawat, M. S., Saraf, S., & Saraf, S. (2007). Phytosome: A novel approach towards functional cosmetics. *Journal of Plant Sciences, 2*, 644–649.

Hakuli, A., Kytokivi, A., Lakomaa, E. L., & Krause, O. (1995). FT-IR in the quantitative analysis of gaseous hydrocarbon mixtures. *Analytical Chemistry, 67*, 1881–1886.

He, X., Li, J., Gao, H., Qiu, F., Cui, X., & Yao, X. (2003a). Six new andrographolide metabolites in rats. *Chemical and Pharmaceutical Bulletin, 51*, 586–589.

He, X., Li, J., Gao, H., Qiu, F., Hu, K., Cui, X., et al. (2003b). Identification of a rare sulfonic acid metabolite of andrographolide in rats. *Drug Metabolism & Disposition, 31*, 983–985.

Hou, D., Xie, C., Huang, K., & Zhu, C. (2003). The production and characteristics of solid lipid nanoparticles (SLNs). *Biomaterials, 24*, 1781–1785.

Humanson, G. L. (1962). *Animal tissue technique*. San Francisco: W.H. Freeman Publisher and Distributor.

Jain, P. K., Khurana, N., Pounikar, Y., Gajbhiye, A., & Kharya, M. D. (2013). Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide vesicular system. *Journal of Liposome Research, 23*, 110–118.

Jung, H. W., Jung, J. K., Ramalingam, M., Yoon, C. H., Bae, H. S., & Park, Y. K. (2012). Antidiabetic effect of wen-pi-tang-hab-wu-ling-san extract in streptozotocin-induced diabetic rats. indian. *Journal of Pharmacology, 44*, 97–102.

Jung, M., Park, M., Lee, H. C., Kang, Y. H., Kang, E. S., & Lim, S. K. (2006). Antidiabetic agents from medicinal plants. *Current Medicinal Chemistry, 13*, 1203–1218.

Madav, H. C., Tripathi, T., & Mishra, S. K. (1995). Analgesic, antipyretic, and antiulcerogenic effects of andrographolide. Indian. *Journal of Pharmaceutical Sciences, 57*, 121–125.

Maiti, K., Mukherjee, K., Gantait, A., Saha, B. P., & Mukherjee, P. K. (2006). Enhanced therapeutic potential of naringenin–phospholipid complex in rats. *Journal of Pharmacy and Pharmacology, 58*, 1227–1233.

Maiti, K., Mukherjee, K., Murugan, V., Saha, B. P., & Mukherjee, P. K. (2010). Enhancing bioavailability and hepatoprotective activity of andrographolide from *andrographis paniculata*; a well known medicinal food, through its herbosome. *Journal of the Science of Food and Agriculture, 90*, 43–51.

Matsud, T., Kuroyanangi, M., Sygiyama, S., Umehara, K., Ueno, A., & Nishi, K. (1994). Cell differentiation-inducing diterpenes from *andrographis paniculata*. *Chemical and Pharmaceutical Bulletin, 42*, 1216–1225.

Meng, Z. M. (1981). Studies on the structure of the adduct of andrographolide with sodium sulfite. *Acta Pharmacologica Sinica, 16*, 571–575.

Mishra, S. P., & Gaikar, V. G. (2006). Aqueous hydroropic solutions as an efficient solubilizing agent for andographolide from *andrographis paniculata* leaves. *Separation Science and Technology, 41*(6), 1115–1134.

Mukherjee, B., Mahapatra, S. K., Das, S., & Patra, B. (2005). Sorbitanmonolaurate 20 as a potential skin permeation enhancer in transdermal patches. *The Journal of Applied Research, 5*, 96–108.

Mukherjee, P. (2002). *Quality control of herbal drugs, an approach to evaluation of botanicals*. New Delhi, India: Business Horizons Pharmaceutical Publisher.

Murugian, P., Palanisamy, M., Stanley, A., & Akbarsha, M. A. (1995). Prospective use of andrographolide in male antifertility. In *Proceedings of the International Symposium on Male Contraception Present and Future* (pp. 34–35).

Nugroho, A. E., Andrie, M., Warditiani, N. K., Siswanto, E., Pramono, S., & Lukitaningsih, E. (2012). Antidiabetic and antihyperlipidemic effect of *andrographi spaniculata* (Burm. f.) nees and andrographolide in high-fructose-fat-fed rats. *Indian Journal of Pharmacology, 44*, 337–381.

Otake, T., Mori, H., Morimoto, L. T., Hattori, M., & Namba, T. (1995). Screening of indonesian plant extracts for anti-human immunodeficiency virus-type 1 (HIV-1) activity. *Phytotherapy Research, 9*, 6–10.

Panossian, A., Hovhannisyan, A., Mamikonyan, G., Abrahamian, H., Hambardzumyan, E., Gabrielian, E., et al. (2000). Pharmacokinetic and oral bioavailability of andrographolide from *andrographis paniculata* fixed combination kan jang in rats and human. *Phytomedicine : international journal of phytotherapy and phytopharmacology, 7*, 351–364.

Parveena, R., Ahmada, F. J., Iqbala, Z., Samimb, M., & Ahmad, S. (2014). Solid lipid nanoparticles of anticancer drug andrographolide: Formulation, *in vitro* and *in vivo* studies. *Drug Development and Industrial Pharmacy, 40*(9), 1206–1212.

Puri, A., Saxena, R., Saxena, R. P., & Saxena, K. C. (1993). Immuno-stimulant agents from *andrographis paniculata*. *Journal of Natural Products, 56*, 995–999.

Qiu, R. L., Li, L., Li, X., Zhang, H. X., & Chen, J. W. (2004). Effect of ultra-fine powder technique on dissolution rates of andrographolide and dehydroandrographolide in *andrographis paniculata*. *Journal of Chinese Integrative Medicine, 2*, 456–458.

Quinten, T., Gonnissen, Y., Adriaens, E., De Beer, T., Cnudde, V., Masschaele, B., et al. (2009). Development of injection moulded matrix tablets based on mixtures of ethylcellulose and low-substituted hydroxypropylcellulose. *European Journal of Pharmceutical Sciences, 37*, 207–216.

Rajagopal, S., Kumar, R. A., Deevi, D. S., Satyanarayana, C., & Rajagopalan, R. (2003). Andrographolide, a potential cancer therapeutic agent isolated from *andrographis paniculata*. *Journal of Experimental Therapeutics and Oncology, 3*, 147–158.

Ren, K., Zhang, Z., Li, Y., Liu, J., Zhao, D., Zhao, Y., et al. (2009). Physicochemical characteristics and oral bioavailability of andrographolide complexed with hydroxypropyl-beta-cyclodextrin. *Die Pharmazie, 64*, 515–520.

Sarwa, K. K., Das, P. J., & Mazumder, B. A. (2014). Nano-vesicle topical formulation

of bhut jolokia (hottest capsicum): A potential anti-arthritic medicine. *Expert Opinion on Drug Delivery, 11*, 661–676.

Sarwa, K. K., Mazumder, B., Rudrapal, M., & Verma, V. K. (2013). Potential of capsaicin-loaded transferosomes in arthritic rats. *Drug Delivery, 25*, 1–9.

Saxena, S., Jain, D. C., Gupta, M. M., Bhakuni, R. S., Mishra, H. O., & Sharma, R. P. (2000). High performance thin layer chromatographic analysis of hepatoprotective dilerpenoids from *andrographis paniculata*. *Phytochemical Analysis, 11*, 34–36.

Sharma, A., Gupta, N. K., & Dixit, V. K. (2010). Complexation with phosphatidylcholine as a strategy for absorption enhancement of boswellic acid. *Drug Delivery, 17*, 587–595.

Sheeja, K., Guruvayoorappan, C., & Kuttan, G. (2007). Antiangiogenic activity of *andrographis paniculata* extract and andrographolide. *International Immunopharmacology, 7*, 211–221.

Shen, Y. C., Chen, C. F., & Chiou, W. F. (2002). Andrographolide prevents oxygen radical production by human neutrophils: Possible mechanism(s) involved in its anti-inflammatory effect. *British Journal of Pharmacology, 135*, 399–406.

Suo, X. B., Zhang, H., & Wang, Y. Q. (2007). HPLC determination of andrographolide in rat whole blood: Study on the pharmacokinetics of andrographolide incorporated in liposomes and tablets. *Biomedical Chromatography, 21*, 730–734.

Verma, V. K., Sarwa, K. K., & Zaman, K. (2013). Antihyperglycemic activity of *swertiachirayita* and *andrographis paniculata* plant extracts in streptozotocin-induced diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*, 305–311.

Widyawaruyanti, A., Asrory, M., Ekasari, W., Setiawan, D., Radjaram, A., Tumewu, L., et al. (2014). *In vivo* antimalarial activity of *andrographis paniculata* tablets. *Procedia Chemistry, 13*, 101–104.

Wongkittipong, R., Prat, L., Damronglerd, S., & Gourdon, C. (2004). Solid–liquid extraction of an from plants—Experimental study, kinetic reaction and model. *Separation and Purification Technology, 40*, 147–154.

Xu, H. W., Dai, G. F., Lui, G. Z., Wang, J. F., & Liu, H. M. (2007). Synthesis of andrographolide derivatives: A new family of α-glucosidae inhibitors. *Bioorganic & Medicinal Chemistry, 15*, 4247–4255.

Xue, Y. M. (2003). Structural study of inclusion complex of andrographolide with β-cyclodextrin prepared under microwave irradiation. *Chinese Chemical Letters, 14*, 155–158.

Yanyu, X., Yunmei, S., Zhipeng, C., & Qinenq, P. (2006). The preparation of silybin–phospholipid complex and the study on its pharmacokinetics in rats. *International Journal of Pharmaceutics, 307*, 77–82.

Yu, B. C., Hung, C. R., Chen, W. C., & Cheng, J. T. (2003). Antihyperglycemic effect of andrographolide in streptozotocin-induced diabetic rats. *Planta Medica, 69*, 1075–1079.

Zhang, A., Sun, H., & Wang, X. (2013). Recent advances in natural products from plants for treatment of liver diseases. *European Journal of Medicinal Chemistry, 63*, 570–577.

Zhang, H., Su, Y., Wang, X., Mi, J., Huo, Y., & Wang, Z. (2017). Antidiabetic activity and chemical constituents of the aerial parts of *Heracleum dissectum* ledeb. *Food Chemistry, 214*, 572–579.

Zhang, X. F., & Tan, B. K. (2000). Anti-diabetic property of ethanolic extract of *Andrographis paniculata* in streptozotocin-diabetic rats. *Acta Pharmacologica Sinica, 21*, 1157–1164.

Zhao, D., Liao, K., Ma, X., & Yan, X. (2002). Study of the supramolecular inclusion of β-cyclodextrin with andrographolide. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 43*, 259–264.

Zhao, L., & Feng, S. (2005). Effects of lipid chain unsaturation and head group type on molecular interactions between paclitaxel and phospholipids within model biomembrane. *Journal of Colloid and Interface Science, 285*, 326–335.