

Extended Release Floating Microballoons Containing *Clerodendrum colebrookianum* Extract: *In vitro in vivo* Evaluation

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ABSTRACT

Aims: Microballoons are floating particulate system comprising of a hollow core, drug being loaded within the polymeric layer. The aim of the study was to prepare microballoons containing extract of *Clerodendrum colebrookianum*, as bioactive agent. **Methods:** The microballoons were prepared by solvent diffusion technique, prepared microballoons were physico-chemically characterized (Scanning Electron Microscopy (SEM), buoyancy determination, entrapment efficiency, etc), *in vitro* drug release study and estimated for its *in vivo* antihypertensive activity by 2K1C hypertension induction method. **Results:** SEM confirmed the microballoons to be spherical with porous surfaces and hollow core. The developed formulation showed a buoyancy varying from 70.18%-89.90%. The entrapment efficiency of bioactives in microballoons was found between 52%-69% with a delayed release of maximum 28% in 8 hr as found from *in vitro* drug release study. *In vivo* antihypertensive studies carried out in rat model showed *C. colebrookianum* extract have good antihypertensive activity in both high and low dose with respect to standard propranolol. The formulations containing both doses were further found to be effectual in the treatment of hypertension because of its floating nature. **Conclusion:** The results suggested that optimized formulation show an effective extended release of *Clerodendrum colebrookianum* extract for the treatment of hypertension.

Key words: Microballoons, Antihypertensive agent, Encapsulation, Floating drug delivery system, 2K1C hypertension induction method.

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INTRODUCTION

Cardiovascular diseases are known worldwide as primary cause of death. It includes Heart attacks, cerebrovascular disease, Hypertension, Periphery artery disease, heart failure, congenital heart disease and Rheumatic heart disease.¹ Hypertension is identified as the continuous increased in pressure in the blood vessels. Hypertension can cause other disorders like rupture of blood vessels, kidney failure, blindness and cognitive impairment.² Treatment of hypertension by consumption of diet containing antioxidant

effect is said to be effective and less costly. Thus consumption of antioxidants which occurs naturally from the daily diet or taken as dietary supplements can help us to maintain a proper metabolic process that are essential for quenching of Reactive Oxygen Species (ROS). The Dietary Approaches to Stop Hypertension (DASH) studies have revealed that blood pressure can be lowered effectively by consuming diet enriched with all these rich nutrients.³ Thereby, use of



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natural sources containing antioxidant activity which are consumed as vegetables are said to be beneficial.

Clerodendrum colebrookianum Walp., belonging to Family Verbenaceae, a medicinal plant used widely by the people of northeast India as a cardio protective agent. It is prevalently known in English as “East Indian Glory Bower”, “Phuinum” in Mizoram, as “Nefafu” in Assam, Khasi as “Jarem”, in Manipur as “Kuthap” and “Phuihong” in Nagaland.⁴ Ethnic population of Mizoram claims that the plant is having a potent hypotensive action when consumed on a regular basis. Phytochemical investigation shown the existence of triacontane, amyirin, clerodin, (24s) ethyl cholesta 5, 22, 25 trien 3-ol, β -sitosterol, clerodendoside, daucosterol, clerosterol, colebrin A-E, clerodolone.⁵ The leaves and leaf twigs are used for treatment of higher blood pressure, abdominal pain, as an anthelmintic, blood purifier, in treatment of infant’s cough and also for the treatment of diabetes by the north-eastern folks of India. The tender stem is used to treat any gastric disorders. People from Mizoram and Arunachal Pradesh used the extract from the roots part for the treatment of diarrhea and dysentery.⁶ Extraction of the plant and then formulated it in a dosage form (microballoons) can increase the efficacy against hypertension and also for ease administration.

Floating drug delivery systems (Microballoons) consist of a central hollow space where the API is loaded within the polymer layer which are developed in such a manner that they have less density than the gastric fluid and can float or continue to float in the stomach, thereby overcoming the drawbacks by increasing the Gastric Residence Time (GRT) or decreased GI transit of the dosage form. The floating mechanisms of such formulations involve swelling and expanding, mucoadhesion and effervescent systems.^{7,8}

The current study was designed with the hypothesis that when *C. colebrookianum* extract was formulated as microballoons, the polymeric matrix will release the potent phytochemical in a sustained manner so as to maintain the effective therapeutic level in the body over an extended period of time and thus eliminating possibility of multiple administrations.

MATERIALS AND METHODS

Materials

Ethylcellulose, Hydroxypropyl methylcellulose [molecular weight: 59.08708; viscosity grade of 2% (w/w) solution: 4-6 cps], Methylcellulose (viscosity: 3000-5000 mPa.s), Polyvinylpyrrolidone (PVP K-30), Polyvinyl alcohol (molecular weight: 140,000), Tween 80, Carboxy meth-

ylcellulose high viscosity (CMC) and Hydrochloric acid were purchased from Himedia, Mumbai. Sodium Chloride was purchased from Qualigens. Aluminium Chloride and Folin-Ciocalteu reagent was purchased from Merck. All other reagents and solvents used were of analytical grade and used without further purification.

Collection and authentication of plant material

The leaves of *Clerodendrum colebrookianum* Walp. were collected from Sivsagar district of Assam, India during the month of June-July and January-February. The leaves were washed thoroughly followed by shade drying and were preserved as herbarium sheet. The authentication was carried out in Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, authentication number being DU/PSC/HRB/DM/1/2016.

Preparation of Plant Extract

The shade dried leaves were grinded to obtain powder passing through sieve no 60 of ASTM series. About 500g of the grounded leaves were taken and extracted using a Soxhlet apparatus at 40°C with ethanol as a solvent. The extraction was continue for 72 hr. The extract was transferred to a petridish and was concentrated to a dry mass with rotary evaporator (BUCHI, Switzerland) followed by lyophilization (IIC, India) at -40°C into a dried mass by lyophilizer.

Phytochemical evaluation of plant extract

Plants do not consist only of carbohydrates, proteins and lipids as chemical components but other secondary metabolites, which gives physiological and therapeutic effects. Therefore, the extracted material was subjected to various preliminary phytochemical screening for the detection of various secondary metabolites. Detection of alkaloid (Mayer’s test, Wagner’s test, Dragendorff’s test, Hager’s test), carbohydrate (Fehling’s test, Molish’s test, Barfoed’s test, Benedict’s test), phenols (Gelatin Test, Ferric Chloride Test), saponins, glycosides (Lead Acetate Test, Borntrager’s test), flavonoids (Magnesium and Hydrochloric acid reduction/Shinoda test, Alkaline reagent test), proteins and amino acids (Millon’s test, Biuret test, Ninhydrin test, Legal’s test, Keller-Killiani test), phytosterols (Liebermann- Burchard’s test), fixed oils (Spot test, Saponification test), fats and gums and mucilages were performed.^{9,10}

Estimation of total phenolic content

The total phenolic content of *C. colebrookianum* extract was estimated by the method adopted by Ordonez et al.¹¹ using Folin-Ciocalteu reagent with slight alterations. The phenolics content of extracts was expressed in Gal-

lic Acid Equivalents (GAE). All samples were analyzed in triplicates.

Estimation of total flavonoids content (aluminum chloride colorimetric assay)

The *C. colebrookianum* extract was diluted to a concentration of 1 mg/mL with ethanol and was further diluted. The diluted extract solution (1 mL) and 1 mL of 2% AlCl₃ solution was taken in a test tube and incubated at room temperature for an hour. The absorbance was analyzed using spectrophotometer (UV-1800 Shimadzu, Japan) at 415 nm. The calibration curve of quercetin (standard compound) was prepared with concentrations between 10 -90 µg/mL. The flavonoids content of extracts was expressed in terms of Quercetin Equivalent (QE).¹² All samples were analyzed in triplicates.

Determination of Antioxidant activity (DPPH radical scavenging activity) of *C. colebrookianum* extract

Radical scavenging activity of *C. colebrookianum* extracts against stable DPPH (1, 1-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. DPPH gets reduced when reacts with an antioxidant compound resulting change in color from deep-violet to light-yellow and was measured at 415 nm on a UV/visible light spectrophotometer (UV-1800 Shimadzu, Japan). The free radical scavenging activity was measured by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) employing the method described by Blois.¹³ 1 mL of 0.1 mM solution of DPPH in ethanol was added to 3 mL of different concentrations (5-100 µg/mL) of the extract and incubated for 30 mins. The percentage of inhibition was calculated by comparing the absorbance of control with absorbance of test samples. Ascorbic acid was used as a reference compound. All the tests were performed in triplicate.

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Preparation of microballoons

With slight modification, preparation of microballoons as reported by Kawashima *et al.*¹⁴ using emulsion solvent diffusion method. Ethyl cellulose was used as a polymer either alone or in mixture with other polymers such as HPMC, Eudragit S-100, Methylcellulose or PVP K-30. At room temperature, 20 mL solution containing ethanol and dichloromethane (1:1) was taken for dissolving the polymers and extract. A solution containing 0.75%w/v polyvinyl alcohol and 0.2 % w/v Tween 80 was prepared at 40°C. Under continuous stirring (mechanical stirrer), the organic solvent was added drop wise to the aqueous solution at a phase volume ratio of 1:5. Three different levels of stirring speed i.e., at 200, 500 and 700 rpm was taken to stir the resultant emulsion continuously. For a better diffusion of solvent in the aqueous phase, the optimum speed selected was 500 rpm. Ultimately leading to the evaporation of organic solvent under stirring. The microballoons formed were collected, washed with distilled water, dried in hot air oven and stored in vacuum desiccators.¹⁵⁻¹⁷ Details reported in Table 1.

Evaluation of microballoons

Percentage Yield of microspheres

Percentage (%) yield was calculated by considering the total non-volatile ingredients used for the preparation of microballoons with the total weight of formed microballoons.¹⁸ The % yield was calculated using the following equation:

$$\% \text{ yield} = \frac{(\text{Weight of microballoons collected})}{(\text{Weight of extract + polymer used for the preparation of microballoons})} \times 100$$

Determination of flow properties of microballoons

Bulk density and tapped density were carried out using Tap density tester (LABINDIA TD1025, INDIA) for the prepared formulation. In a graduated measuring cylinder (10 mL), 1g of formulation was weigh, trans-

Table 1: Composition table for the experimental formulations.

Formulation Code	Extract	EC*	HPMC#	PVP**	E##	MC***	EtOH:CH ₂ Cl ₂ ^^
MC1	100mg	2.3g	-	-	-	-	1:1
MC2	100mg	2 g	0.3 g	-	-	-	1:1
MC3	100mg	2 g	-	0.3 g	-	-	1:1
MC4	100mg	2 g	-	-	0.3 g	-	1:1
MC5	100mg	2 g	-	-	-	0.3 g	1:1

*EC- Ethyl cellulose, #HPMC- Hydroxypropyl methylcellulose, **PVP- Polyvinylpyrrolidone K-30, ##E- Eudragit S-100, ***MC- Methylcellulose, ^^EtOH: CH₂Cl₂ - Ethanol: dichloromethane

ferred and the volume was noted.¹⁸ Followed by the determination of Carr's index and Hausner's ratio.¹⁹ The angle of repose was studied using fixed funnel method. Microballoons were poured through a funnel onto a graph paper where the tip was fixed at a minimum height of 2cm above the cone. The angle of repose is the \tan^{-1} ratio of the height of the cone and radius of its base.¹⁸

Determination of buoyancy

Microballoons (50 mg) were transferred to a 100 mL Simulated Gastric Fluid (SGF), pH 1.2 comprising 0.02% Tween 20 (to prevent aggregation of microballoons) at 37°C. At 100 rpm the mixture was stirred using a magnetic stirrer. After 8 h, the microballoons (floated and settled) were collected and separated, dried at 40°C and weighed. The buoyancy was calculated by the equation given below:²⁰

$$\text{Buoyancy (\%)} = \frac{(\text{Weight of floating microballoons})}{(\text{Weight of floating microballoons} + \text{Weight of settled microballoons})} \times 100$$

Determination of entrapment efficiency and drug loading

C. colebrookianum extract was diluted to different concentration 20, 40, 60, 80, 100 and 120 µg/ml for the standard calibration curve of flavonoids present in extract. 1 ml of AlCl₃ solution (2%) was added to the diluted solution (1mL), incubated for an hour at room temperature. The absorbance was measured using UV-spectrophotometer (UV1800, Shimadzu) at 415 nm. Microballoons (10mg) were crushed, added to ethanol to dissolve the main components and filtered. The filtrate was taken for measurement to determine the absorbance by a UV-spectrophotometer (UV1800, Shimadzu) at wave length of 415 nm.²¹ Entrapment efficiency and drug loading was calculated using following formulae:

$$\text{Entrapment efficiency (\%)} = \frac{(\text{Practical drug content})}{(\text{Theoretical drug content})} \times 100$$

$$\text{Drug loading} = \frac{(\text{Amount of drug recorded})}{(\text{Amount of formulation taken})} \times 100$$

Scanning electron microscopy (SEM)

External morphology of the prepared microballoons was analyzed by SEM both before and after release study. Using a double adhesive tape, the microballoons

were mounted onto the aluminium stub. Using Jeol JFC 1100 sputter coater the stub was then coated with gold. The microballoons were observed at 15 kV accelerating voltage.¹⁶ The analysis was performed by using Jeol JSM 6360, Japan.

Infra-red Spectroscopy

An IR Spectrum reveals the distinguishing peaks of the sample functional groups. For ascertaining any possible chemical interaction of extract, polymer, their physical mixture and extract loaded microballoons were exposed to FTIR studies. IR spectra were recorded using IR spectrophotometer (Shimadzu model 8400), between 500 cm⁻¹ to 4000 cm⁻¹ wavenumbers.¹⁶

In vitro drug release and release kinetics studies

The release profile from different formulation was determined using USP type II apparatus. Dissolution medium (SGF, pH 1.2, 900 mL) containing 0.02% Tween 20 was filled in the dissolution vessel, temperature maintained at 37±0.5°C and stirred at 100 rpm. A weight amount of microballoons equivalent to 50 mg of flavonoid was transferred to the dissolution vessel containing the dissolution medium. At a predetermined time intervals, aliquots were withdrawn till the 8th hour. Samples were then analyzed by UV spectrophotometer (UV 1800, Shimadzu) at 415 nm.²² Data obtained after the *in vitro* drug release were statistically fitted to several kinetic models to determine the drug release kinetics for the microballoons.

Antihypertensive activity

In vivo Antihypertensive activity of *C. colebrookianum* extracts as well as the microballoons formed were carried out using of male wistar albino rats (*Rattus albus*), weighing 150-300g. The *in vivo* pharmacodynamic study was approved and certified (vide approval No. IAEC/DU/117, Dated 18/02/2016) by the Institutional Animal and Ethics Committee, Department of Pharmaceutical Sciences, Dibrugarh University (Reg No. 1576/GO/E Re/S/11/CPCSEA, Dated 23/12/2014), Dibrugarh, Assam, India.

Induction of Two kidney one clip (2K1C) renal hypertension

The rats were acclimatized for 7 days post which they were anesthetized with Ketamine HCl, 40 mg/kg (intra-peritoneal injection). The experimental procedure was conducted under aseptic conditions. The animal left posterior side was shaved followed by sterilization with 70% ethanol. An incision was performed on the left flank to give entry to the left renal artery followed by isolation from the renal vein and connective tissue. The

exposed renal artery was applied with silver clip with diameter (internal) of 0.2 mm, which was turned with the goal that the opening slit facing the abdomen, bringing about fractional occlusion of the renal perfusion. The opposite kidney was not disturbed. After surgery, sutured the muscles and skin immediately, the rats were then allowed to recuperate from anesthesia. A sham treatment for the control group was achieved by undergoing the same procedure as 2K1C rats, except for the application of a clip. Animals were allowed to access 8% NaCl solution.^{23,24} Blood pressure were measured by non-invasive tail cuff plethysmography method (Biopac system NIBP 200A, MP 36) and were considered hypertensive when the systolic pressure was over 160 mmHg. The rats of different groups were treated with oral administration of Propanolol (Standard drug), extract of *C. colebrookianum* with a dose of 100mg/kg b.w. (CC100) and 200mg/kg b.w. (CC200) and the formulation with a dose equivalent to 10mg/kg flavonoid.^{25,26} The blood pressure was monitored over a period of 7 days and reported.

Statistical Analysis

The data presented here is for triplicate determinations ($n=3$) and it is expressed as mean \pm SD. Antihypertensive activity data were investigated by One-way ANOVA, using Graph pad INSTAT. The post-hock analysis was performed by Dunnet's multiple comparison tests to evaluate the significance difference between individual groups (** $P<0.01$). Confidence interval has been considered as 99% and $p < 0.01$ were considered significant. IC_{50} value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

RESULTS AND DISCUSSION

Phytochemical evaluation of plant extracts

Phytochemical test revealed the constituents present which are well known for their vivid medicinal and physiological activities. The phytochemical evaluation of the ethanolic extracts were performed and the results were reported in Table 2.

Estimation of Total phenolic content

The total phenolic content for *C. colebrookianum* extract using the Folin-Ciocalteu's reagent was found to be 621.32 mg of GAE/g.

Estimation of Total Flavonoid Content

The flavonoid content for *C. colebrookianum* extract was found to be 539.7 mg of Quercetin equivalent/g. In

Table 2: Phytochemical screening of ethanolic extract of *C. colebrookianum*.

Test	Result
Detection of Alkaloids	Positive
Detection of Carbohydrates	Positive
Detection of Saponins	Positive
Detection of Phenolic Compounds	Positive
Detection of Glycoside	Positive
Detection of flavonoids	Positive
Detection of Proteins and Amino Acids	Negative
Detection of Phytosterols	Positive
Detection of Fixed Oils and Fats	Negative
Detection of Gums and Mucilage	Negative

brief, the flavonoid present in the extract reacts with the aluminium ion (Al^{3+}) to form the stable flavonoid- Al^{3+} complex, which has a yellow color and whose intensity is related to the concentration of flavonoid present in it. Flavonoids which are supposed to be the key components for demonstration of anti-hypertensive activity was confirmed by the test^{27,28} although other classes of compounds also may contribute for the blood pressure normalization to demonstrate a synergistic effect. Therefore it is expected that the extract would show a considerable reduction in blood pressure when formulated in a sustained release dosage form.

Antioxidant Activity: DPPH radical scavenging activity of *C. colebrookianum* extract

In DPPH free radical scavenging activity was performed and observed that there is an increased in scavenging effect with increase concentration of *C. colebrookianum* extract. It was also observed that at same concentration ascorbic acid expressed the highest % inhibition i.e. 94.56% than *C. colebrookianum* extract with 73.75%, with IC_{50} of 123.21 μ g/mL for standard, whereas for extract it was 133.44 μ g/mL. Hence, *C. colebrookianum* can be used as good source of antioxidant which may be an important factor to efficiently lower the blood pressure, by decreasing the aldehyde conjugate/AGE formation resulting in reduced oxidative stress and enhancing insulin resistance and other endothelial functions.^{3,29-31}

Preparation of Microballoons

The microballoons containing *C. colebrookianum* extract were prepared by emulsion solvent diffusion method using ethyl cellulose as polymer either alone or on mixture with other polymers (composition details were reported in Table 1) to manipulate the formulation yield, encapsulation/loading efficiency and release

kinetics of bioactive extract in the formulation. The central hollow core of microballoons is most likely due to the difference in diffusion rate and miscibility of ethanol and dichloromethane in aqueous phase. In the primary stage of microballoons formation, ethanol diffuses readily from the aqueous phase due to its high miscibility followed by the slow diffusion of dichloromethane leading to the development of the central hollow space filled with water. This water was evaporated from the cavity through the porous surface while drying at controlled temperature, eventually forming hollow microballoons.³² The development of microballoons had been confirmed in further evaluation studies.

Evaluation of Microballoons

The Percentage yield, Bulk density, Tapped density, Carr's index, Hausner Ratio and Angle of repose of all formulations were reported in the Table 3. MC1 have the lowest yield with MC3 showing the highest product yield which may attribute to the binding capacity of PVP. The lower the value of Carr's index better will be the flow properties, values higher than 25 is said to give a poor flowability.³³ The results show that MC1 and MC4 exhibit a good flow and the others i.e. MC2, MC3 and MC5 have a fair flow property. Hausner's ratio of 1.25 or less is said to give good flow ability of materials.³⁴ The MC2 showed a poor flow properties while all the others give a good flow properties. In the present study, the angle of repose varies from 20.02°C–33.36°C depending on nature of formulation. Overall after characterizing the flow property, the angle of repose values of all micro balloons were acceptable as free-flowing as they did not exceed 35°C.

Floating / Buoyancy Test

All microspheres showed a floating ability or buoyancy varying between 70.18% – 89.90% up to 8 hr (Table 3). The buoyancy is credited to the air core hollow structure

of microballoons formed after the evaporation of solvent. Floation studies indicate that all the formulation had good buoyancy. MC3 showed the highest % buoyancy.

Entrapment efficiency and Drug Loading

The formulations were calculated for drug entrapment efficiency and loading using equation 4 and 5 respectively. From Table 3, revealed that MC3 showed entrapment efficiency better than the other, which may thus be inferred that ethyl cellulose along with PVP to formed a uniform and effective outer layer. Drug loading remained almost similar in all the five formulations.

Compatibility study: FTIR Spectroscopy

From Figure 1, FTIR of *C. colebrookianum* extract, ethyl cellulose, PVP K-30, physical mixture and MC3 formulation revealed a similar characteristic peaks. Thus it can be concluded that there is no possible chemical interaction between the extract and the excipients and it indicates the integrity and compatibility of the polymer and the extract.

Scanning Electron Microscopy study

SEM images of microballoons provide information about particle shape, size and external morphology of the formulations. Microballoons were observed to be spherical and porous in nature with central hollow core as showed in Figure 2, which were accountable for the floating behaviour. The pores on the surface were observed to increase in diameter after dissolution study confirming the theory of diffusion from insoluble matrix to be the mechanism of drug release.

In vitro Drug release study and Release order kinetics study

The *in-vitro* drug release studies from all the formulations revealed an early burst release of flavonoid from microballoons, followed by gradual retarded release of

Table 3: Physicochemical and *in vitro* evaluation of microballoons.

Formulation	MC1	MC2	MC3	MC4	MC5
Percentage of Yield (%)	47.43±0.02	58.30±0.2	68.45±0.015	63.15±0.005	58.99±0.118
Bulk density (gm/cm ³)	0.072±0.001	0.088±0.001	0.13±0.005	0.072±0.001	0.13±0.005
Tapped density (gm/cm ³)	0.081±0.001	0.113±0.001	0.16±0.005	0.082±0.005	0.158±0.001
Carr's Index (%)	11.11±0.010	22.22±0.010	20±0.550	12.27±0.010	17.98±0.020
Hausner's ratio	1.125±0.001	1.28±0.010	1.25±0.005	1.139±0.001	1.219±0.001
Angle of repose (θ)	29.57±0.005	30.26±0.010	29.83±0.010	32.62±0.005	27.79±0.005
Buoyancy Test (%)	78.26±0.025	77.94±0.020	89.90±0.064	75.55±0.052	80.69±0.032
Entrapment Efficiency (%)	52.09±0.005	63.40±0.100	81.3±0.100	68.28±0.010	69.31±0.020
Drug Loading (%)	3.38±0.005	3.35±0.01	3.65±0.01	3.33±0.025	3.62±0.037

Values are expressed as Mean ± SD; where n = 3.

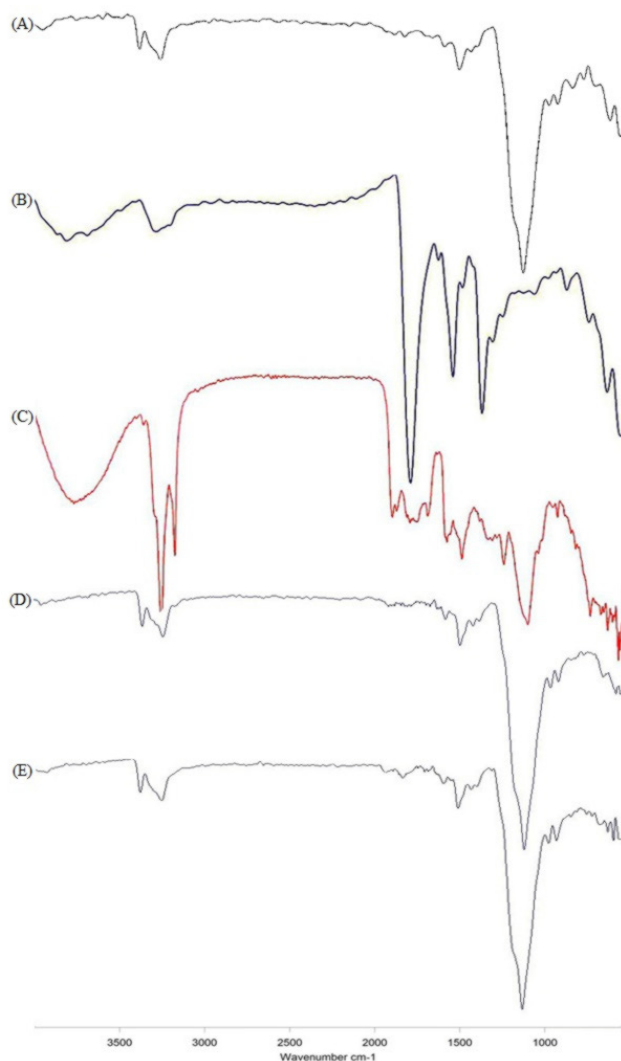


Figure 1: Compatibility study by FTIR spectrum: (A) Ethylcellulose (B) PVP K-30 (C) *C. colebrookianum* extract (D) physical mixture of Ethylcellulose + PVP K-30 + *C. colebrookianum* extract (E) MC3 formulation.

flavonoid in stimulated gastric fluid, ranging between 1.15-28.09%, with MC3 formulation gives better release of flavonoid (28.09% for 8 hr) compared to others as shown in Figure 3. MC3 *in-vitro* release studies data were fitted to several release kinetics models to understand its drug release mechanism. The highest regression was obtained for Higuchi (0.981) followed by first order (0.9641), zero order (0.9536) then Hixon Crowell (0.6694). To determine the release kinetics of the prepared formulations, the dissolution data was analyzed using korsmeyer-Peppas equation. Here, after the values of percentage of cumulative drug release are fitted to the equation and it was observed that MC3 formulation followed super case-II transport. From the release kinetic study, release of flavonoid from the microballoon MC3 was observed to follows Higuchi Model which

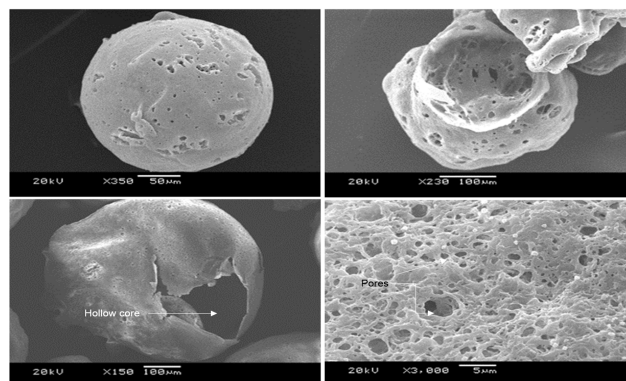


Figure 2: Scanning Electron Microscopy (SEM) images of microballoons displaying their spherical shape, centre hollow and porous nature.

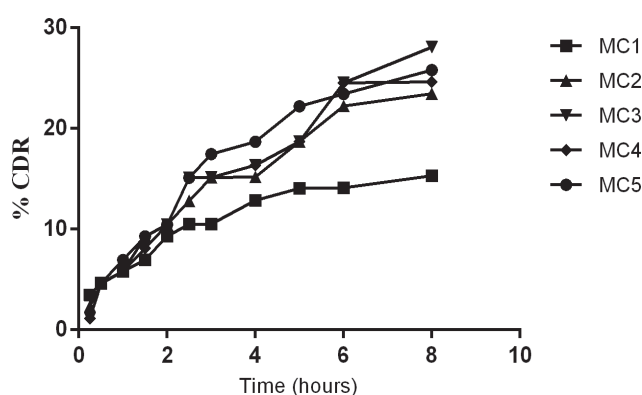


Figure 3: *In vitro* release of flavonoids from experimental microballoons.

describes that the uniform dispersion of drug in matrix media and drug release rate is associated to the drug diffusion rate.

Antihypertensive activity of *C. colebrookianum* extract and Microballoons

The activity of *C. colebrookianum* extracts as well as prepared extract loaded microballoons was checked using *in vivo* animal model. From Table 4 and Figure 4, it can be said that *C. colebrookianum* extract showed activity at both levels of dosing (100mg/kg b.w. and 200mg/kg b.w.), where the high dose gives a better activity than the low dose. The oral administration of formulation MC3 demonstrated better antihypertensive activity after dosing than the crude extract. This may be because of increased gastric retention of extract when it was formulated as floating microballoons, resulting in enhancement of bioavailability of active molecule although one tenth of the extract dose was administered. The efficacy to reduce the arterial blood pressure by the *C. colebrookianum* extract and propranolol confirms the potency of extract to be safe in treatment of hypertension. This fact may be credited to the presence

Table 4: In vivo evaluation of antihypertensive activity of extract (CC100 and CC200) and extract loaded formulation (MC3).

Particulars	Positive control	Negative control	Standard	CC 100	CC 200	MC3
Before treatment (Compared with control)	99.66±2.074	94.40 ±4.35	91.39 ±1.57**	97.30 ± 2.759	93.39 ±7.24*	94.38 ±1.91 *
Before treatment (Compared with standard)	99.66 ±2.0**	94.40 ±4.3	91.39 ±1.5	97.30 ±2.7*	93.39 ±7.2	94.38 ±1.9
After treatment (Compared with Control)	99.52 ±1.1	139.61±11.7**	88.11 ±1.9**	94.54 ±2.13	91.79 ±5.8 *	92.16 ±1.6*
After treatment (Compared with standard)	99.52 ±1.17**	139.61±11.71**	88.11 ±1.93	94.54 ±2.13	91.79 ± 5.8	92.16 ±1.66

Values are expressed as Mean ± SD., where $n = 6$. One Way ANOVA followed by Turkey-Kramer Multiple Comparison test

*Less significant; **Moderately significant where $p < 0.01$ vs. control group / standard group

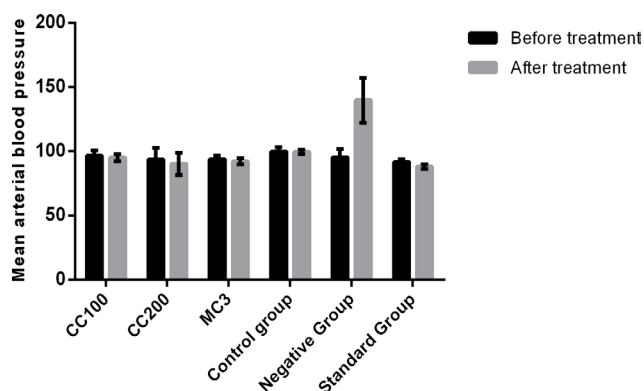


Figure 4: In vivo antihypertensive study in rat model.

of antioxidants like flavonoid present in the extract. Reduction in blood pressure in standard group and both the test groups was found significant when compared with negative control group.

CONCLUSION

In summary, successful development of extended release antihypertensive microballoons containing *C. colebrookianum* extract were effectively prepared and evaluated. It was additionally confirmed from the *in vivo* studies, that extract loaded MC3 microballoons were effective in the management of hypertension. In future, with intense and insightful study, *C. colebrookianum* extract can be processed to isolate pure bioactive compound responsible for producing the anti-hypertensive activity. The potent bioactive molecule can then be further formulated as novel formulations for example microballoons or any other sustained release system for safe and effective management of hypertension.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

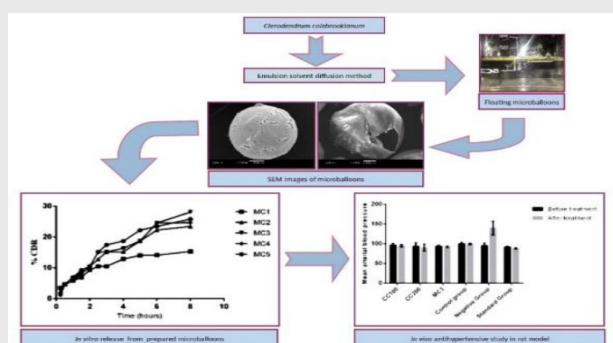
ROS: Reactive oxygen species; **DASH:** Dietary Approaches to Stop Hypertension; **GRT:** Gastric Residence Time; **CMC:** Carboxy methylcellulose, **GAE:** Gallic acid equivalents; **QE:** Quercetin equivalent; **DPPH:** 1, 1-diphenyl-2-picrylhydrazyl hydrate; **SGF:** Simulated gastric fluid; **SEM:** Scanning electron microscopy; **FT-IR:** Fourier Transforms-Infra Red; **2K1C:** Two kidney one clip.

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PICTORIAL ABSTRACT



SUMMARY

In this study the crude extract from the leaf *Clerodendrum colebrookianum* Walp. a traditionally known antihypertensive plant which was formulated into microballoons a floating drug delivery system. The basic idea behind the development of such a system is to achieve a sustained release thus maintaining a constant level of drug in the blood plasma. The novelty of the work is combination of traditional system of medicine with novel drug delivery system.

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