AAPS PharmSciTech, Vol. 10, No. 4, December 2009 (© 2009)
DOI: 10.1208/s12249-009-9329-8

Research Article

Intranasal Cabergoline: Pharmacokinetic and Pharmacodynamic Studies

Gitanjali Sharma,[1] Anil Kumar Mishra,[2] Pushpa Mishra,[2] and Ambikanandan Misra[1,3]

Received 5 December 2008; accepted 13 October 2009; published online 6 November 2009

Abstract. Aims of this investigation were to prepare and characterize cabergoline intranasal microemulsion formulations, determine brain drug delivery through biodistribution using technetium-99m (^{99m}Tc) as a tracer, and assess its performance pharmacodynamically in weight control. Cabergoline microemulsions of different compositions were prepared by water titration method and characterized for globule size and zeta potential. Microemulsion with maximum drug solubilization and stability was considered optimal and taken for further studies with or without addition of mucoadhesive agent. Pharmacokinetics of optimized ^{99m}Tc-labeled cabergoline formulations and ^{99m}Tc-labeled drug solution were studied by estimating radioactivity in brain and blood of albino rats post intranasal, intravenous, and oral administrations. To confirm localization of drug in brain following intranasal, intravenous, and oral administrations, gamma scintigraphy imaging was also performed. To assess weight control performance of formulations, body weight, white adipose tissue mass, serum lipids, leptin, and prolactin were determined before and after 40 days of intranasal administrations of these formulations to Wistar rats. Microemulsions were found to be stable both physically and chemically when stored at various stress conditions. Brain/blood uptake ratios, drug targeting efficiency, and direct drug transport were found to be highest for drug mucoadhesive microemulsion followed by drug microemulsion and drug solution post-intranasal administration compared to intravenous drug microemulsion. Significant ($p<0.05$) reduction in assessed pharmacodynamic parameters was observed after intranasal administration of mucoadhesive microemulsion against control group. The results of the studies conclusively demonstrate that intranasal microemulsion formulations developed in this investigation are stable and can deliver cabergoline selectively and in higher amounts to the brain compared to both drug administrations as a solution intranasally or microemulsion intravenously. The results also demonstrate reduction in weight, adipose tissue mass, serum lipids, and serum prolactin after intranasal administration of drug microemulsion. Hence, long-term studies in at least two more animal models followed by extensive clinical evaluation can safely result into a product for clinical use.

KEY WORDS: biodistribution; cabergoline; intranasal; obesity; radiolabeling.

INTRODUCTION

Cabergoline (Cab) is a potent semi-synthetic ergot alkaloids used for the treatment of diseases caused by hyperprolactinemia (11) (elevated serum prolactin levels >20 μg/L) and few neurological disorders (11) (Parkinson's disease) as dopamine agonist. However, review of recent research suggests its possible application as a lipid lowering and anti-obesity agent by controlling serum prolactin levels. In a study on golden hamsters (8), it was observed that reduced prolactin retards liver lipogenesis even if insulin is available in abundance for supplying glucose for lipogenesis. In a human study covering

[1] Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Kalabhavan, Post Box No.51, Vadodara, 390 001, Gujarat, India.
[2] Ministry of Defence, Institute of Nuclear Medicine and Allied Sciences, Government of India, Brig. S.K. Mazumdar Marg, Timarpur, Delhi, 110054, India.
[3] To whom correspondence should be addressed. (e-mail: misraan@hotmail.com, misraan@satyam.net.in)

ABBREVIATIONS: BBB, Blood–brain barrier; BMI, Body mass index; Cab, Cabergoline; CME1, Cabergoline microemulsion containing polyethylene glycol 400 as cosurfactant; CME2, Cabergoline microemulsion containing transcutol as cosurfactant; CMME11, Cabergoline mucoadhesive microemulsion containing polyethylene glycol 400 as cosurfactant and chitosan as mucoadhesive agent; CMME12, Cabergoline mucoadhesive microemulsion containing polyethylene glycol 400 as cosurfactant and polycarbophil as mucoadhesive agent; CMME21, Cabergoline mucoadhesive microemulsion containing transcutol as cosurfactant and chitosan as mucoadhesive agent; CMME22, Cabergoline mucoadhesive microemulsions containing transcutol as cosurfactant and polycarbophil as mucoadhesive agent; CNS, Central nervous system; CS, Cabergoline solution; DTE (%), Drug targeting efficiency; DTP (%), Brain drug direct transport percentage; GSH, Reduced glutathione; HDL, High-density lipoprotein; i.n., Intranasal; i.v., Intravenous; LDL, Low-density lipoprotein; PBS, Phosphate-buffered saline; p.o., Peroral; WHO, World Health Organization; VLDL, Very-low-density lipoprotein; 99mTc-CS, Radiolabeled (99m-technetium) cabergoline solution.; 9mTc-CME1, Radiolabeled (99m-technetium) cabergoline microemulsion containing polyethylene glycol 400 as cosurfactant; 99mTc-CMME11, Radiolabeled (99m-technetium) cabergoline mucoadhesive microemulsion containing polyethylene glycol 400 as cosurfactant and chitosan as mucoadhesive agent; 99mTc, Technetium.

1530-9932/09/0400-1321/0 © 2009 American Association of Pharmaceutical Scientists

overweight subjects (16) bearing prolactin secreting adenomas, normalization in body weight was observed when prolactin levels returned to normal. In another human study, it was observed that the people taking Cab for treating hyperprolactinemia experienced weight loss (20). Cab lowers serum prolactin levels by acting on dopaminergic (D_2) receptors in central nervous system (CNS) (11). Also, it has been reported that the body has a homeostatic mechanism for controlling body fat and weight through CNS (14). Flier (14) had summarized many possible pathophysiological mechanisms involved in the development and maintenance of body fat and weight. This field of research had not been approached until leptin was discovered in 1994. Leptin (17,18) and other appetite-related hormones act on the hypothalamus, a region of the CNS, to regulate food intake and energy expenditure. Thus, CNS is the key target for drugs controlling body fat and weight by regulating lipogenesis.

It was hypothesized that intranasal delivery of Cab may be more useful than the intravenous, oral, vaginal, or transdermal administration due to enhanced patient compliance by virtue of reduced side effects (11), reduced drug dose and dosing frequency, and probably, better body fat and weight control. All these drug administration routes suffer from one common limitation, that the CNS uptake of the drugs from the systemic circulation is limited by the presence of BBB, which is not the case with intranasal delivery (23). This drug delivery route works because of the unique neuronal connection which the trigeminal and olfactory nerves provide between the nasal cavity and the cerebrospinal fluid and brain. Intranasal micoremulsions have been reported to provide a noninvasive technique for direct nose to CNS drug delivery (22–24,27), and a mucoadhesive microemulsion consisting of mucoadhesive polymer provides prolonged residence in the nasal cavity (2,36,37). It is desirable to achieve rapid and complete absorption of drugs. Also, microemulsions are suitable carriers for drugs susceptible to hydrolysis in aqueous medium like Cab (32). Hence, an attempt was made to develop an intranasal formulation to deliver Cab directly to CNS to control body fat and weight, maximize therapeutic index of the drug, and reduce associated side effects (26).

Thus, the objectives of the study were to prepare, optimize, and characterize microemulsion containing Cab for intranasal administration, to assess its distribution in CNS using ^{99m}Tc as a tracer, and to ascertain its performance pharmacodynamically on rats in controlling body fat and weight. It was also hypothesized that a Cab-containing mucoadhesive microemulsion will deliver Cab in higher amounts to the nigrostriatal and tubero-hypophyseal regions (frontal cortex region) of CNS via the olfactory and trigeminal neuronal pathways and will act on dopaminergic neurons there to exert the weight control activity by inhibiting prolactin secretion.

MATERIALS AND METHODS

Materials

Cabergoline was given as a gift by Pfizer, Groton, CT. Capmul MCM-L8 was given as a gift by Abitech Corporation Limited, Columbus, OH. Labrafac lipo, Labrafil 2125, Labrasol, and Transcutol were given as a gift by Colorcon Asia Private Ltd., Goa, India. Chitosan 652 was purchased from Siber Hegner India Pvt. Ltd., Mumbai and polycarbophil (Noveon AA1) was given as a gift by BF Goodrich Company, Akron, OH. Other chemicals were of analytical grade. Water used in all the studies was distilled and filtered through 0.22-μm nylon filter before use.

Microemulsion Preparation and Characterization

The cabergoline solution (CS, 0.167% *w/w* cabergoline) was prepared by dissolving Cab in acetate buffer pH 5 with stirring. The drug-loaded microemulsions (CME, 0.167% *w/w* Cab) were prepared by completely dissolving Cab in a mixture of oil Capmul MCM L8 (O, 4.4% *w/w*), surfactant Tween-80 (S, 24% *w/w*), and co-surfactant (CoS) polyethylene glycol 400 (CME1, 6% *w/w*) or transcutol (CME2, 6% *w/w*). Acetate buffer pH 5 (AQ) was added gradually with continuous stirring to obtain transparent and homogenous Cab microemulsions CME1 and CME2 (Table I), respectively (transmittance at 630 nm >98%). No heating was conducted during the preparation of microemulsions.

The mucoadhesive microemulsions (CMME11 and CMME21) were prepared by adding chitosan solution (1% *w/w* in acetate buffer pH 5) with stirring to the continuous phase such that the final content of chitosan in the formulations is 0.5% *w/w*. Similarly, mucoadhesive microemulsions CMME12 and CMME22 were prepared by adding polycarbophil solution (1% *w/w* in distilled water, adjusted to pH 5) with stirring to the continuous phase such that final content of polycarbophil in the formulations is 0.5% *w/w*. No heating was conducted during the preparation of mucoadhesive microemulsions. Cab was estimated (7) using UV-Visible spectroscopy (Shimadzu 1601, Japan) at 280 nm against methanol as blank after confirming the noninterference of excipients in the absorbance region of Cab.

The drug-loaded microemulsions and mucoadhesive microemulsions were characterized for globule size (30,37) (Nano ZS, Malvern Instruments), zeta potential (30,37) (Nano ZS, Malvern Instruments), assay (Shimadzu 1601, Japan), pH (Systronics 335, India), viscosity (34) (Brookfield HADV III+) conductivity (34) (Conductometer CM 180 Elico, India), and nasal toxicity (39) using excised sheep nasal mucosa, mucosa treated with PBS pH 6.4 and isopropyl alcohol were taken as positive and negative control, respectively (Fig. 1).

In Vitro Drug Release Studies

To elucidate the effect of microemulsion and mucoadhesive microemulsion systems on release kinetics of Cab, release studies were performed for CS, CME1, and CMME11 in acetate buffer pH 5 using dialysis method. The cellulose acetate membrane (molecular weight cutoff=12,000 kDa) was hydrated in the buffer solution for 24 h. One end of pretreated cellulose dialysis tubing (7 cm in length) was tied with thread, and then 0.6 mL of each formulation was placed in it along with 1 mL of dialyzing medium. The other end of the tubing was also secured with thread and was allowed to rotate freely in 75 mL of dialyzing medium and stirred continuously at 100 rpm with magnetic bead on magnetic plate at 37°C. Aliquots of 0.6 mL were removed at different time intervals and diluted further with methanol. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for Cab dialyzed

Table I. Composition and Characterization[a] of Microemulsions

Test	CS	CME1	CME2	CMME11	CMME21	CMME12	CMME22
				Cab mucoadhesive microemulsions			
	Cab solution	Cab microemulsions		Chitosan (0.5%*w/w*)		Polycarbophil (0.5%*w/w*)	
O (%)	–	4.4	4.4	4.4	4.4	4.4	4.4
S (%)	–	24	24	24	24	24	24
CoS (%)	–	6 (P)	6 (T)	6 (P)	6 (T)	6 (P)	6 (V)
AQ (%)	100	65.6	65.6	65.6	65.6	65.6	65.6
Assay (%*w/w*)	99.5±0.05	102.2±0.10	98.5±0.09	101.3±0.11	99.6±0.05	99.2±0.12	98.8±0.16
pH	5.8±0.12	6.7±0.13	6.78±0.09	5.75±0.11	5.9±0.12	5.33±0.08	5.25±0.12
Conductivity (mS)	–	0.228±0.09	0.182±0.07	3.21±0.08	3.42±0.12	0.194±0.11	0.123±0.12
Viscosity (Cp)	–	246.2±0.56	245.6±0.63	287.2±0.49	285.8±0.58	264.2±0.45	268.1±0.60
Zeta potential (mV)	–	-7.9±1.20	-6.82±2.80	10.8±2.50	13.7±2.90	-17.5±3.10	-18.2±3.30
Globule size (nm)	–	32.2±3.30	24.9±4.60	29.9±7.50	37.1±8.80	29.5±7.90	32.1±9.10
Radiolabeled complex (%)	98.7±0.12	98.9±0.13	–	97.3±0.11	–	–	–
Stability Study							
Assay (%*w/w*)	–	98.1±0.12	–	98.3±0.21	–	–	–
pH	–	6.3±0.13	–	5.55±0.11	–	–	–
Zeta potential (mV)	–	-1.9±2.60	–	6.8±3.50	–	–	–
Globule size (nm)	–	38.2±5.30	–	36.0±8.50	–	–	–

O oil (Capmul MCM L8), *S* surfactant (polysorbate 80), *CoS* co-surfactant, *P* polyethylene glycol-400, *T* for transcutol, *AQ* aqueous phase (acetate buffer pH 5)
[a] The results are mean values ± SEM derived from three different experimental batches. The formulations contain Cab 1.67 mg/mL

across the membrane using UV-visible spectrophotometer (Shimadzu 1601, Japan) at 280 nm against methanol as blank. The cumulative amount of Cab released and release coefficient/flux across cellulose membrane was calculated for the formulations (Table II). The kinetics of Cab from the test formulations was evaluated by fitting the experimental data to different order kinetics such as zero-order, first order, and Higuchi's model. Each experiment was repeated three times.

In Vitro Drug Diffusion

The *in vitro* drug diffusion study (38) was performed using Franz diffusion cell of diameter 10 mm mounted with excised sheep nasal mucosa (39) of thickness (height) 0.2 mm. CS, CME1, CME2, CMME11, CMME12, CMME21, and CMME22 were placed in the donor compartment and recipient compartment contained 12 ml of acetate

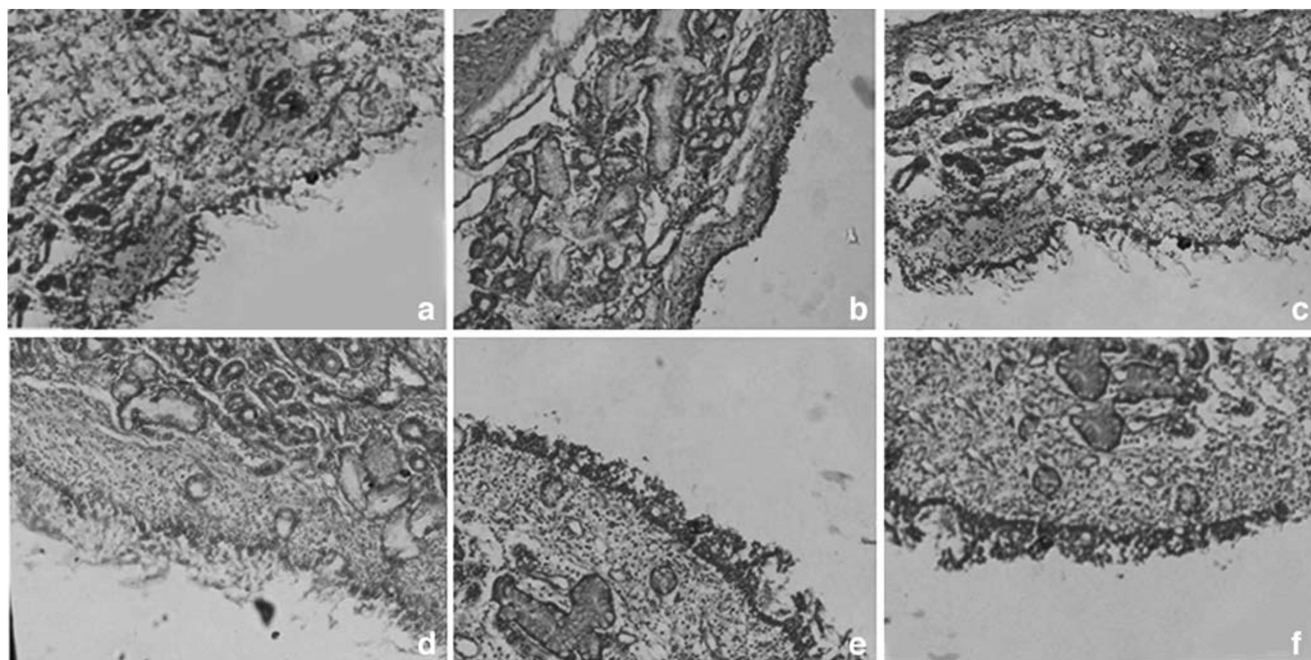

Fig. 1. **a** Nasal mucosa treated with PBS-6.4, **b** Nasal mucosa treated with isopropyl alcohol, **c** Nasal mucosa treated with CME1, **d** Nasal mucosa treated with CME2, **e** Nasal mucosa treated with CMME1, **f** Nasal mucosa treated with CMME12. Optical microscopy of Cab formulation-treated sheep nasal mucosa to access nasal toxicity

Table II. Results of *In-vitro* Drug (Cab) Diffusion Across Sheep Nasal Mucosa and *In Vitro* Drug Release Studies Across Dialysis Membrane

Time (min)	Percentage drug released (% *w/w*)			Percentage drug diffused (% *w/w*)						
	CS	CME1	CMME11	CS	CME1	CME2	CMME11	CMME21	CMME12	CMME22
0	0	0	0	0	0	0	0	0	0	0
5	–	–	–	22.59±0.056	17.72±0.871	31.55±0.756	16.73±0.879	17.3±0.234	21.0±0.819	18.19±0.230
15	44.7±0.236	27.32±0.145	24.83±0.215	25.51±0.121	19.44±1.160	31.62±0.987	28.39±0.786	37.09±0.658	28.5±0.567	18.52±0.356
30	52.1±0.252	37.25±0.147	29.8±0.310	44.68±0.376	20.65±0.765	31.81±0.878	33.79±0.767	46.28±0.766	32.95±0.379	18.97±0.274
45	–	–	–	50.1±0.564	23.28±0.980	31.9±1.198	41.88±0.896	50.8±0.496	38.16±0.983	19.23±0.867
60	59.6±0.148	49.67±0.287	44.7±0.219	57.3±0.786	26.8±1.150	31.97±1.090	51.8±1.987	57.51±1.620	40.41±1.156	19.58±0.356
90	84.4±0.326	57.44±0.273	53.19±0.302	66.23±1.156	28.43±1.120	32.09±2.450	54.4±1.109	58.65±0.598	46.35±2.320	20.5±1.145
120	94.3±0.193	74.22±0.279	67.05±0.224	68.13±0.976	29.44±2.080	32.21±1.150	66.3±1.897	59.57±2.190	46.35±1.540	23.06±2.150
150	–	–	–	70.38±2.134	37.67±1.980	32.57±2.950	73.98±2.286	60.18±3.090	46.35±3.110	23.5±0.957
180	96.8±0.251	80.7±0.160	76.99±0.273	70.55±1.978	45.67±2.890	32.87±3.870	96.56±2.981	62.5±1.143	46.35±0.892	24.13±0.987
210	–	–	–	70.62±1.121	57.67±2.360	33.12±1.980	97.42±1.786	64.57±0.753	46.35±2.180	25.13±2.347
240	101.31±0.183	95.8±0.135	90.37±0.194	–	–	–	–	–	–	–
Diffusion coefficient/ Release coefficient	0.351±0.102	0.348±0.034	0.337±0.067	0.2754±0.097	0.1949±0.098	0.0554±0.035	0.4114±0.086	0.2175±0.078	0.1507±0.059	0.063±0.049

Values are expressed as mean ± SEM of three estimations

buffer pH 5 stirred with Teflon coated magnetic stirrer (120 rpm). Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed spectrophotometrically (7). Each sample removed was replaced with an equal volume of acetate buffer pH 5. Each study was carried for a period of 3.5 h and in triplicate. The mean cumulative values for percentage drug diffused and diffusion coefficients for Cab were calculated for the formulations (Table II).

Stability Studies

Formulations CME1 and CMME11 were subjected to stability study for a period of 2 months at ambient conditions (30°C/ 60RH). After 2 months of storage, the microemulsions were subjected to test for physical stability (22) for any drug precipitation or phase separation on accelerated centrifugation cycle (3,000 rpm for 15 min), drug assay, pH, globule size, and zeta potential determination.

Pharmacokinetics

Radiolabeling of Formulations

CS, CME1, CMME11, CMME12, and placebo of each were radiolabeled using technetium-99m (^{99m}Tc) by direct labeling method (12,36). To 1 ml of each formulation, stannous chloride dihydrate solution (200 μg in 100 μL of 0.10 N HCl) was added, and the pH was adjusted to 6.80± 0.20 using 50 mM sodium bicarbonate solution. To the resultant mixture, required volume of sterile ^{99m}Tc-pertecnetate (35 to 40 mCi/ml) was added with continuous mixing such that the resultant solution has a radioactivity of 2.5 to 3 mCi/ml and incubated at 30±5°C for 10 min. The final volume was made up to 1.5 ml using 0.9% *w*/*v* sterile sodium chloride solution.

The radiochemical purity (3,35,40) of ^{99m}Tc-CS (^{99m}Tc-labeled CS), ^{99m}Tc-CME1 (^{99m}Tc-labeled CME1), ^{99m}Tc-CMME11 (^{99m}Tc-labeled CMME11), and ^{99m}Tc-CMME12 (^{99m}Tc-labeled CMME12) were determined using ascending instant thin layer chromatography. The effect of incubation time, pH, and stannous chloride concentration on radiolabeling efficiency (3,31,37) were studied to achieve optimum reaction conditions. *In vitro* stability of radiolabeled formulations in 0.9% *w*/*v* sodium chloride (15,37) (normal saline) was also evaluated.

Biodistribution Studies

With prior approval from The Social Justice and Empowerment Committee, Ministry of Government of India, animal experiments were conducted, for the purpose of control and supervision on animals and experiments. Swiss albino rats (male, aged 4 to 5 months), weighing between 150 and 200 g, were selected for the study, and three rats for each formulation per time point were used in the study. Radiolabeled complex of ^{99m}Tc-Cab formulations (100 μCi/50 μL to 150 μCi/50 μL) containing 0.045±0.002 mg/kg body weight Cab was administered (10 μL) in each nostril. The rats were held from back in slanted position, and formulations were instilled into nostrils with the help of micropipette (10

to100 μL) attached with low-density polyethylene tubing, having 0.1 mm internal diameter at the delivery site. Similarly, 20 μL of radiolabeled complex of ^{99m}Tc-CME1 (100 μCi/50 μL to 150 μCi/50 μL) containing 0.045±0.002 mg/kg bodyweight Cab was injected through tail vein of Swiss albino rats. The rats were killed humanely at different time intervals and the blood collected using cardiac puncture. Subsequently, brain and other tissues were dissected, washed twice with normal saline to remove adhering tissue/fluid, and weighed. Radioactivity present in each tissue/organ was measured using shielded well-type gamma scintillation counter. Radiopharmaceutical uptake per gram in each tissue/organ was calculated as a fraction of administered dose (3,37) using following equation and tabulated in Table III:

$$\frac{\%\ \text{Radioactivity}}{\text{gm of tissue}} = \frac{\text{counts in sample} \times 100}{\text{wt of sample} \times \text{total counts injected}} \quad (1)$$

The brain concentrations of Cab *versus* time (hour) plot for the formulations are shown in Fig. 2. Pharmacokinetic parameters were calculated using Quickcal software and recorded in Table IV. Brain targeting efficiency was assessed based on the values of drug targeting efficiency (DTE%) (37,41), which represents time average partitioning of drug between brain and blood, and brain drug-direct-transport percentage (DTP%) (37,41), which represents the percentage of drug directly transported to the brain through the olfactory and trigeminal neural pathway and were calculated using the following equations and tabulated in Table IV.

$$\text{DTE\%} = \frac{\left[\left(\frac{AUC_{brain}}{AUC_{blood}}\right)_{in}\right]}{\left[\left(\frac{AUC_{brain}}{AUC_{blood}}\right)_{iv}\right]} \times 100 \quad (2)$$

Where, AUC indicates area under the curve.

$$\text{DTP\%} = \frac{B_{in} - B_x}{B_{in}} \times 100 \quad (3)$$

Where,

$$B_x = \left(\frac{B_{iv}}{P_{iv}}\right) \times P_{in} \quad (4)$$

Nomenclature

B_x	Brain AUC fraction contributed by systemic circulation through the blood–brain barrier (BBB) following intranasal administration.
B_{iv}	$AUC_{0\to240}$ brain following intravenous administration.
P_{iv}	$AUC_{0\to240}$ blood following intravenous administration.
B_{in}	$AUC_{0\to240}$ brain following intranasal administration.
P_{in}	$AUC_{0\to240}$ blood following intranasal administration.
AUC	Area under the curve.

Gamma Scintigraphy Imaging

Swiss albino rats (150 to 200 g, male) were selected for the study. Radiolabeled formulation of ^{99m}Tc-CME1 (100 μCi/50 μL to 150 μCi/50 μL) containing 0.045±0.002 mg/kg BW Cab was injected through tail vein of Swiss albino rats. Radiolabeled formulations ^{99m}Tc-CS/CME1/CMME11/CMME12 (100 μCi/50 μL to 150 μCi/50 μL) were administered (10 μL) in each nostril similarly as described under biodistribution study. The rats were anesthetized using diazepam subcutaneous injection prior to administration of formulations. The anesthetized rats were placed on board, and images were captured using single positron emission computerized tomography (SPECT, LC 75-005, Diacam,

Table III. Compartmental Distribution of ^{99m}Tc-CME1 (i.v.), ^{99m}Tc-CS (i.n.), ^{99m}Tc-CME1 (i.n.), ^{99m}Tc-CMME11 (i.n.), and ^{99m}Tc-CME1 (p.o.) at Predetermined Time Intervals in Normal Swiss Albino Rats

Formulation (route of administration)	Distribution of Cab in blood and brain at predetermined time intervals					
	Organ	0.25 h	0.5 h	1 h	2 h	4 h
CME1 (i.v.)	Blood	0.01±0.003	0.021±0.003	0.054±0.005	0.071±0.015	0.06±0.006
	Brain	0.00032±0.00004	0.00056±0.00005	0.0021±0.00040	0.002±0.00050	0.0016±0.00040
CS (i.n.)	Blood	0.006±0.00060	0.007±0.00040	0.0094±0.00040	0.0083±0.00070	0.0073±0.00050
	Brain	0.0025±0.0006[a]	0.0032±0.0002[a]	0.0037±0.0005[a]	0.0033±0.0003[a]	0.0026±0.0005[a]
CME1 (i.n.)	Blood	0.011±0.00300	0.023±0.00400	0.029±0.00500	0.021±0.00400	0.014±0.00400
	Brain	0.0028±0.00040[a,b]	0.0042±0.00060[a,b]	0.0046±0.00040[a,b]	0.0035±0.00040[a,b]	0.0031±0.00020[a,b]
CMME11 (i.n.)	Blood	0.014±0.00300	0.022±0.00250	0.013±0.00310	0.007±0.00200	0.009±0.00220
	Brain	0.0031±0.00030[a,b]	0.0053±0.00050[a,b]	0.0065±0.00060[a,b]	0.0061±0.00050[a,b]	0.0048±0.00040[a,b]
CME1 (p.o.)	Blood	0.0011±0.00030	0.002±0.00050	0.0024±0.00040	0.0021±0.00020	0.0013±0.00040
	Brain	0.0	0.0	0.00002±0.000004	0.00008±0.00002	0.00003±0.000001
CME1 (i.v.)	Brain/Blood	0.032±0.0700	0.027±0.0020	0.039±0.0030	0.028±0.0007	.0027±0.0040
CS (i.n.)	Brain/Blood	0.417±0.0600[a]	0.457±0.04[a]	0.362±0.03[a]	0.398±0.0500[a]	0.371±0.0700[a]
CME1 (i.n.)	Brain/Blood	0.255±0.0500[a]	0.183±0.0300[a]	0.159±0.0230[a]	0.167±0.0350[a]	0.221±0.0700[a]
CMME11 (i.n.)	Brain/Blood	0.221±0.0440[a]	0.241±0.0090[a]	0.5±0.0750[a]	0.871±0.1200[a]	0.53±0.1000[a]
CME1 (p.o.)	Brain/Blood	0.0	0.0	0.0083±0.0005	0.038±0.0020	0.023±0.0040

Abbreviations are explained in Table I. The rats were administered with 100 μCi ^{99m} Tc-Cab, and the radioactivity was measured in percent per gram of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations

[a] Variation in values between CS (i.n.), CME1 (i.n.), or CMME11 (i.n.) when compared to CME1 (i.v.) or CME1 (p.o.) are significant ($p<0.05$)

[b] Variation in values between CME1 (i.n.) or CMME11 (i.n.) when compared to CS (i.n.) are significant ($p<0.05$)

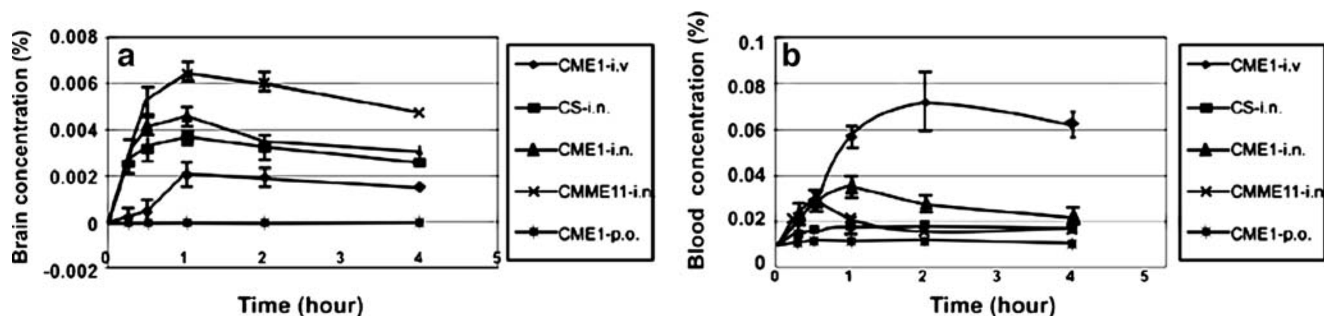

Fig. 2. **a** Brain concentrations *versus* time plot following administrations of ^{99m}Tc-Cab formulations. **b** Blood concentrations *versus* time plot following administrations of ^{99m}Tc-Cab formulations

Siemens AG, Erlanger, Germany) gamma camera (3,6). The scintigraphy images after 0.5 h following intravenous and intranasal administrations of formulations are shown in Fig. 3.

Pharmacodynamic Studies

The anti-obesity activity of Cab was estimated in Wistar albino rats (either sex, 150 to 250 g) (25,36), following intranasal administration (body weight Cab) of 10 μL of CMME11 (0.167% *w/w* Cab), twice a week, using micropipette (10 to100 μL) attached with low-density polyethylene tubing, having 0.1 mm internal diameter at the delivery site. The study was carried out for a period of 40 days and the results recorded in Table V.

Induction of Obesity in Animals

The albino rats were fed with high-fat diet of composition 0.5% *w/w* cholesterol 10% *w/w* butter, 10% *w/w* coconut oil, 0.1% *w/w* sodium cholate, and 79.4% *w/w* normal rodent pellet diet for a period of 10 weeks, to induce obesity. The body weight and serum lipid levels (estimated in 10–12-h-fasted animals) of high-fat-diet-fed animals (in comparison to control group fed with only normal rodent pellet diet) were carefully estimated, both at the initiation and at the end of 10 weeks, and recorded. The estimation was carried out using Monozyme Liquichem Cholesterol, Monozyme India Ltd, Secunderabad for serum total cholesterol and HDL cholesterol and Triglycerides Liquid, Reckon Diagnostics Pvt. Ltd., Baroda, Gujarat, India, for serum triglycerides (25,35).

Evaluation of Anti-Obesity Activity

Group I Control—not receiving any drug but received placebo formulation intranasally
Group II Treated—received CMME11 intranasally

Estimation of white adipose tissue mass. The two abdominal white adipose tissue depots—infrarenal white adipose tissue and gonadal white adipose tissue (either periovarian fat in female rat or epididymal fat in male rat)—were removed from killed animals, washed with saline, quickly blotted on filter paper, and weighed. The mass of white adipose tissue (WAT) was expressed as % *w/w* of total animal body weight (35,36).

Estimation of serum prolactin. The serum prolactin was estimated by a chemiluminescence assay on an Advia Centaur automated system (Bayer Advia, NY)

Estimation of serum leptin. The serum leptin was estimated as per the procedure given in radioimmunoassay kit of Linco, Seaford, DE.

Estimation of serum lipid profile. The estimation was carried out using Monozyme Liquichem Cholesterol, Monozyme India Ltd, Secunderabad for serum total cholesterol and HDL cholesterol, and Triglycerides Liquid, Reckon Diagnostics Pvt. Ltd., Baroda, Gujarat, India, for serum triglycerides.

Statistical analysis. All data are reported as mean ± SEM, and the difference between the groups were tested using Student's *t* test at the level of $p<0.05$, and differences greater at $p<0.05$ were considered insignificant.

RESULTS AND DISCUSSION

The Cab microemulsions CME1 and CME2 and mucoadhesive microemulsions CMME11, CMME12, CMME21, and CMME22 were prepared by water titration method (Table I) and characterized for globule size (24.9±4.6 to 37.1±8.8 nm), zeta potential (−18.2±3.3 to 13.7±2.9 mV), assay (98.8±0.16% to 102.2±0.1%), pH (5.25±0.12 to 6.78±0.09), viscosity (245.6±0.63 to 287.2±0.49 Cp), and conductivity (0.123±0.12 to 3.42±0.12 mS), and the results are recorded in Table I. Addition of chitosan or polycarbophil as mucoadhesives had no effect on the globule size of Cab microemulsions but affected the pH, viscosity, conductivity, and zeta potential of microemulsions by virtue of their inherent properties. Chitosan being a cationic polymer contributed positively and polycarbophil being an anionic polymer contributed negatively to the zeta potential of mucoadhesive microemulsions which is thought to improve the stability of formulation by preventing aggregation. These polymers being mucoadhesive improved the viscosity of Cab microemulsions from 245.6±0.63 to 287.2±0.49 Cp needed for increasing the residence time of the formulation in nasal cavity. The microemulsions as expected had a Newtonian flow behavior with respect to shear rate and shear stress while mucoadhesive microemulsions being polymeric in nature had non-Newtonian flow behavior. Polycarbophil was found to have no appreciable effect on the conductivity of formulations

Table IV. Pharmacokinetics of ^{99m}Tc-CME1 (i.v.), ^{99m}Tc-CS (i.n.), ^{99m}Tc-CME1 (i.n.), ^{99m}Tc-CMME11 (i.n.), and ^{99m}Tc-CME1 (p.o.), at Predetermined Time Intervals in Swiss Albino Rats [a] and Drug Targeting Efficiency and Direct Nose-to-brain Transport Following Intranasal Administration of ^{99m}Tc-CS, ^{99m}Tc-CME1, and ^{99m}Tc-CMME11

Formulation and route of administration	Organ/tissue	C_{max} (%/g)	T_{max} (h)	$AUC_{0\to240}$ (h^{a}%/g)	$AUC_{0\to\infty}$ (h^{a}%/g)	K_{el} (L/h)	$T_{1/2}$ (h)	Drug targeting efficiency (%DTE)	Direct nose-to-brain transport (%DTP)
CME1 (i.v.)	Blood	0.071±0.0150	2±0.15	0.2174±0.030	0.393±0.030	0.342±0.040	2.0284±0.410	100±0.0	–
	Brain	0.0021±0.0004	1±0.10	0.0065±0.001	0.011±0.003	0.3555±0.090	1.9495±0.960		
CS (i.n.)	Blood	0.0094±0.0004	1±0.10	0.0309±0.002	0.122±0.040	0.0801±0.015	1.61±0.670	2,566.7±828.320	83.65±3.280
	Brain	0.0037±0.0005	1±0.10	0.0122±0.001	0.0337±0.083	0.1208±0.06	2.22±0.710		
CME1 (i.n.)	Blood	0.029±0.0050	1±0.10	0.0786±0.015	0.1234±0.031	0.3129±0.080	2.2146±0.360	1,033.3±2.780	92.57±0.630
	Brain	0.0046±0.0004	1±0.10	0.0141±0.001	0.0356±0.004	0.1438±0.030	3.175±0.660		
CMME11 (i.n.)	Blood	0.022±0.0025	0.5±0.05	0.041±0.008	0.0662±0.014	0.3572±0.057	1.94±0.470	2,766.7±212.680	94.35±1.210
	Brain	0.0065±0.0006	1±0.10	0.0216±0.0017	0.0553±0.0053	0.1424±0.014	4.87±0.410		
CME1 (p.o.)	Blood	0.0024±0.0004	1±0.10	0.0073±0.001	0.012±0.005	0.276±0.140	2.5112±1.260	70±1.256	Value in negative
	Brain	0.00008±0.00002	2±0.15	0.0002±0.00003	0.0009±0.00003	0.0397±0.028	1.796±0.12		

[a] The rats were administered with 100 µCi *^{99m}*Tc-Cab and the radioactivity was measured in percent per g of tissue of the administered dose. Values are expressed as mean ± SEM of three estimations

but chitosan increased the conductivity of microemulsions from 0.182±0.07 to 3.42±0.12. It may contribute to drug permeation across nasal mucosa as it has been reported (19,34) that skin permeation also increases on application of potential. The pH of the mucoadhesive microemulsions was observed to be shifted from 6.78±0.09 to 5.25±0.12, on incorporation of chitosan or polycarbophil, making it more biocompatible intranasally as normal physiological pH of human nasal mucosa is being reported to be between 4.5 and 6.5. The formulation pH reported to have profound effect on the mucoadhesive property of polymers used. Chitosan (21) having a pKa of 6.5 is more soluble and, hence, has better mucoadhesion at formulation pH (~5.5), while polycarbophil (10) having a pKa of 4.2 is less soluble and hence demonstrates poor mucoadhesion at formulation pH. This will further affect drug diffusion across nasal mucosa. The optical microscopy of Cab formulations treated excised sheep nasal mucosa is shown in Fig. 1, and it illustrates no appreciable nasal toxicity of formulations when compared to isopropyl treated sheep nasal mucosa (39).

The results of *in vitro* drug release studies of Cab across cellulose membrane are recorded in Table II. The release coefficient of Cab across cellulose membrane was found to be highest for CS (0.351±0.102) followed by CME1 (0.348±0.034) followed by CMME11 (0.337±0.067). The higher affinity of Cab into the lipophilic internal phase and presence of surfactants in CME1 and CMME11 may have resulted in slower release rate and flux (29). The presence of mucoadhesive polymer in CMME11 may have further decreased the release rate by increasing the viscosity of system (33) when compared to CME1. The formulations CS, CME1, and CMME11 were observed to follow Higuchi's kinetics when fitted into various order kinetics as had highest R^2 values of 0.928, 0.991, and 0.995, respectively, with Higuchi's model. To have a better idea of *in vivo* performance of the developed formulations and to select the most appropriate formula, *in vitro* drug diffusion studies were performed across excised sheep nasal mucosa which closely simulates the biological barrier the formulations will come across during the biodistribution or pharmacodynamic studies. It represents both intracellular and intercellular pathways. The results of *in vitro* drug diffusion studies of Cab across excised sheep nasal mucosa are also recorded in Table II. CME1 and CMME11 among microemulsions and mucoadhesive microemulsions, respectively, were found to possess the highest drug diffusion coefficients (0.1949±0.098 and 0.4114±0.086, respectively) and percentage drug diffused (57.67±2.36% and 97.42±1.786%) across sheep nasal mucosa (39). Although both chitosan and polycarbophil have the same mechanism of enhancing drug permeation across mucosa (by transient widening of the tight junctions within mucosa), chitosan (21), having better solubilization at formulation pH compared to polycarbophil, has better mucoadhesion and, hence, explains the increased drug absorption across sheep nasal mucosa from CMME11 when compared to polycarbophil (CMME12). Also, when compared to *in vitro* drug release studies, the diffusion coefficient of Cab from CMME11 across sheep nasal mucosa was highest than CME1 and CS which can be attributed to the mucoadhesion conferred by chitosan and opening of the tight junctions on interaction with biological membrane. Hence, the microemulsions CME1 and CMME11 were selected for pharmacokinetic studies further.

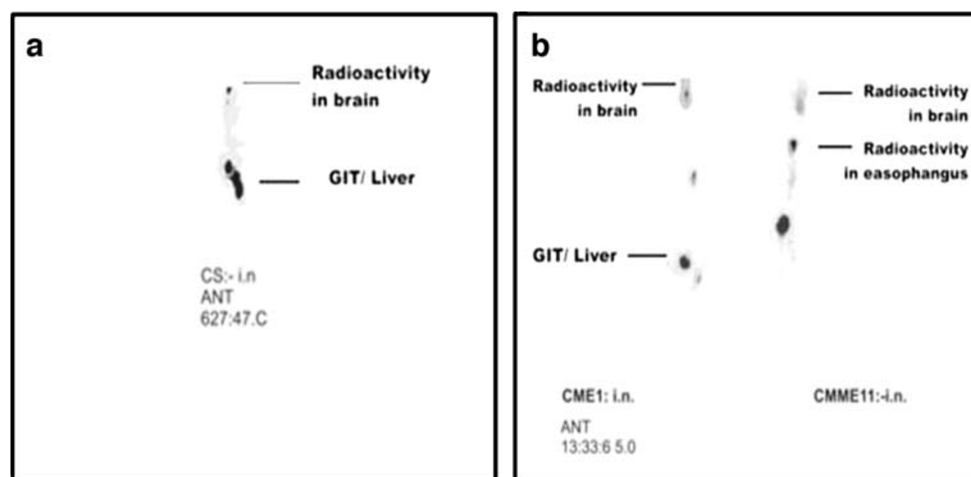

Fig. 3. **a**, **b** Gamma scintigraphy images of rat (A/P view) showing the presence of radioactivity into the brain

CME1 and CMME11 were found to be stable physically (22) as no phase separation or drug precipitation on centrifugation and no change in globule size were observed before and after centrifugation (Table I). The drug assay was observed between 98.1±0.12% and 98.3±0.21% when subjected to storage at ambient conditions (30°C/60RH) for 2 months.

CS, CME1, and CMME11 formulations were effectively radiolabeled with Technetium-99m (^{99m}Tc), optimized for maximum labeling efficiency and stability *in vitro* in saline. Radiochemical purity observed was 98.7%, 98.9%, and 97.3% for CS, CME1, and CMME11, respectively, when evaluated for bound ^{99m}Tc and free ^{99m}Tc. The radiochemical purity for the placebo formulations was found to be less than 5%. Thus, the formulation excipients did not interfere with the radiolabeling of Cab. The optimal $SnCl_2.2H_2O$ concentration was observed to be 200 μg/mL at pH between 6.0 and 7.0 with an incubation time of 10 min. ^{99m}Tc-CS/CME1/CMME11 were found to be stable in normal saline solution up to 8 h (degradation <2% *w/w*). Thus, these formulations, by being stable and having high radiolabeling efficiency, were found suitable for biodistribution studies in albino rats.

Biodistribution studies (36) of ^{99m}Tc-Cab formulations following i.v. (CME1), p.o. (CME1), and i.n. (CS, CME1 and CMME11) administrations in Swiss albino rats were performed, and the radioactivity was estimated at predetermined time intervals up to 4 h. The results obtained are recorded in Table III and shown graphically in Fig. 2. The higher brain concentrations of Cab following i.n. administrations of CME1 and CMME11 than CS may be ascribed to the permeation enhancing effect of surfactants (4,28) and mucoadhesive polymer (21,36). Also, the concentrations of Cab in brain following i.n. administration of CME1 and CMME11 were found to be significantly ($p<0.05$) higher (0.0046±0.0004 and 0.0065±0.0006 μCi) at all sampling time points when compared to i.n. CS and i.v. (0.0021±0.0004 μCi) and p.o. (0.00008±0.00002 μCi) administration of CME1. The brain/blood ratio of the drug at all time points for the formulations were also calculated and recorded in Table III. The brain/blood ratios at 0.25 h for CME1 (i.n.) and CMME11 (i.n.) were found to be 10-fold higher compared to CME1 (i.v.). This may be attributed to the direct nose-to-brain transport of drug following intranasal administration. At time points 0.25 and 0.5 hr, although the brain/blood ratio of Cab was higher for CS than CME1 and CMME11, it declined gradually after 1 h indicating clearance of drug from brain. However, after 1 h, the brain/blood ratio of Cab increased progressively for both CME1 and CMME11 demonstrating improved residence time of drug in brain compared to i.n. CS desirable for drug to have therapeutic effect for a prolong period of time. The increased brain $T_{1/2}$ of drug when administered intranasally ($T_{1/2}$=3.175±0.66 h) compared to intravenous ($T_{1/2}$=1.9495±0.96 h) demonstrates that Cab concentrations in the brain will be sustained for a long time (2 to 4 h) as evident from the plateau-like curve of Fig. 2, which is desirable for drug to have therapeutic effect for a prolong period of time and is not the case with intravenous administration. Also, brain $T_{1/2}$ of Cab changed with formulations in the order drug solution<microemulsion< mucoadhesive microemulsion. The results of biodistribution studies (Table III) demonstrated that the amount of Cab (in brain) from mucoahdesive microemulsion were highest at all time points, followed by drug microemulsion followed by drug

Table V. Results of Anti-Obesity Activity of Cab in Food-Induced Obese Wistar Rats

Parameters	Group I	Group II	% Change
n	6	6	–
Body weight (gm)	335±45.91	297.5±37.07	−11.2
Total cholesterol (mg/dl)	117.05±4.87	97.55±8.20[a]	−16.7
Triglycerides (mg/dl)	147.075±6.37	119.525±9.09	−18.7
HDL (mg/dl)	45.28±1.47	43.33±0.77	−4.31
LDL (mg/dl)	41.8±4.43	27.82±5.70[a]	−33.4
VLDL (mg/dl)	29.42±1.27	23.905±1.82	−18.75
Serum leptin (μg/ml)	2.65±0.20	4.1±0.39[a]	+54.7
Serum prolactin (ng/ml)	0.00475±0.0014	0.00133±0.00071[a]	−72.1
WAT (% w/w)	4.59±0.82	2.66±0.52	−42.19

Values are expressed as mean ± SEM of three observations
[a] Variation between Group I and Group II are significant ($P<0.05$)

solution. Hence, the drug therapeutic concentrations in brain are expected to be maintained for a prolonged period of time with mucoadhesive microemulsion compared to microemulsion or solution resulting in $T_{1/2}$ in same order (drug solution< microemulsion<mucoadhesive microemulsion). This may be attributed to the presence of surfactants in microemulsion system acting as permeation enhancers by reducing interfacial tension across the mucosal membrane (4,28) and the prolonged dilation of tight junctions of nasal mucosa by the mucoadhesive polymer (21) (chitosan) felicitating paracellular drug transport also. It is also supported by the results of *in vitro* drug diffusion studies across sheep nasal mucosa where highest drug flux was observed for mucoadhesive microemulsion. The diffusion of Cab from CMME11 and CME1 continued beyond 4 h compared to CS where a saturation like condition was observed after 2 h.

The pharmacokinetic parameters such as C_{max}, $AUC_{0\to240}$, $AUC_{o\to\infty}$, T_{max}, K_{el} (L/h), and $T_{1/2}$ (h) were calculated using Quickcal software and recorded in Table IV and shown in Fig. 2. Both CME1 (i.n.) and CMME11 (i.n.) show 2- and 50-folds higher C_{max} (brain) and 3- and 40-folds higher AUC (brain) compared to CME1 (i.v.) and CME1 (p.o.), respectively. The drug targeting efficiency [DTE (%)] and brain drug direct transport percentage [DTP (%)] were also calculated for intranasally administered formulations and are recorded in Table IV. The CMME11 showed the highest DTE (%) and DTP (%) values among all the three formulations. Higher DTE (%) and DTP (%) for CMME11 compared to CS may be attributed to the longer residence time of formulation by being more viscous and mucoadhesive and suggests greater extent of selective transport of Cab to the brain.

In order to confirm improved brain uptake following i.n. and i.v. administrations of ^{99m}Tc-Cab formulations, gamma scintigraphy imaging was performed in albino rats 0.5 h post i.v. and i.n. administrations and are shown in Fig. 3. Significantly high radioactivity was noticed in the rat brain for CMME11 (i.n.) compared with CME1 (i.v.) and CME1 (i.n.) and supports selective transport of drug to the brain following intranasal administration. Hence, the observations are consistent with the results recorded in Table III.

To assess the anti-obesity activity of intranasal Cab microemulsions (CMME11), the parameters estimated were body weight, percentage white adipose tissue, serum lipid profile, serum leptin and prolactin levels, and results are recorded in Table V. Cab being unstable in aqueous medium (32), mucoadhesive microemulsion was selected for assessing the anti-obesity activity of intranasal Cab. A noticeable reduction was observed in body weight (11.2±42.26%), while a significant ($p<0.05$) reduction was observed in white adipose tissue mass (42.19±0.67%) and serum total cholesterol (16.7±6.23%) in Group II animals compared to Group I animals receiving intranasal CCME 11 and placebo, respectively. The results (Table V) suggest a possible role of Cab and CCME 11 in control of obesity and reduction in weight of obese patients. These findings are further supported by a significant ($p<0.05$) increase (54.7±0.31%) in serum leptin and a significant ($p<0.05$) decrease (72.1± 0.0019%) in serum prolactin levels associated with decreased food intake. These findings confirm that Cab exerts its anti-obesity activity though modulation of serum leptin and prolactin.

CONCLUSIONS

The intranasal microemulsion of Cab having good physical and chemical stability with no appreciable nasal toxicity was successfully developed by water titration method in this investigation. Mucoadhesive microemulsion (CMME11) was found to have best *in vitro* diffusion across sheep nasal mucosa and also demonstrated 3- and 40-folds higher brain uptake compared to microemulsion administered by intranasal and per-oral routes, respectively. The increased brain $T_{1/2}$ and high DTE (%) and DTP (%) compared to intravenous illustrated selective nose to brain transport of Cab. Pharmacodynamic studies demonstrated profound decrease in body weight, adipose tissue mass, serum lipids, and prolactin when investigated *in vivo* in rats. Studies conducted in this investigation conclusively demonstrate possible role of mucoadhesive microemulsion in control of weight and reduction of weight in obese patients. However, long-term studies in at least two more animal models followed by extensive clinical evaluation can only give a product for clinical use.

Since cab is used in disorders such as hyperprolactinemia (11), dementia and parkinsonism (11), the developed formulation can also find application in treatment of these diseases and may also demonstrate advantage over conventional formulation (tablet) by being more brain selective drug delivery and possibility of reducing drug dose and/or frequency of dosing and, hence, possibly the cost of therapy for treating the diseases. It may be noted due to changed dosage regime in these diseases the possibility of reduction in weight is negligible and lower systemic side effects. However, therapeutic benefits of intranasal Cab over conventional therapies in these disorders need preclinical and clinical studies.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. RP Tripathi, Director, Institute of Nuclear Medicine and Allied Sciences, Ministry of Defense, New Delhi, for providing the necessary facilities to carryout the radiolabeling experiments, Mr. Donnie Owens, Pfizer Inc., Groton for providing gift sample of Cabergoline, Mr. Asis Jain (Abitec Corp. Ltd., Ohio, US) and Ms. Samantha Colaco (Colorcon, India) for providing excipients.

REFERENCES

1. Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB. Annual deaths attributable to obesity in the United States. JAMA. 1999;282(16):1530–8.
2. Alpar HO, Somavarapu S, Atuah KN, Bramwell VW. Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery. Adv Drug Deliv Rev. 2005;57 (3):411–30.
3. Babbar AK, Singh AK, Goel HC, Chauhan UPS, Sharma RK. Evaluation of ^{99m}Tc labeled Photosan-3, a haematoporphyrin derivative, as a potential radiopharmaceutical for tumor scintigraphy. Nucl Med Biol. 2000;27:419–26.
4. Bali V, Bhavna, Ali M, Baboota S, Ali J. Potential of microemulsions in drug delivery and therapeutics: a patent review. Recent Pat Drug Deliv Formul. 2008;2(2):136–44.
5. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospec-

tively studied cohort of U.S. adults. N Engl J Med. 2003;348 (17):1625–38.

6. Capala J, Barth RF, Bailey MQ, Fenstermarker RA, Marek MJ, Rhodes BA. Radiolabeling of epidermal growth factor with 99mTc and *in vivo* localization following intracerebral injection in to normal and glioma bearing rats. Bioconjug Chem. 1997;8:289–95.
7. CDER New Drug Approvals. http://www.fda.gov/cder/foi/nda/index.htm.
8. Cincotta AH, Meier AH. Prolactin permits the expression of a circadian variation in lipogenic responsiveness to insulin in hepatocytes of the golden hamster. J Endocrin. 1985;106 (2):173–6.
9. Cincotta AH, Bina KG. Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing hormone, body weight gain, and hyperglycemia in ob/ob mice. Neuroendocrinology. 2000;71:68–78.
10. Cooper SL, Bamford CH, Tsuruta T. Polymer biomaterials in solution, as interfaces and as solids; polyanionic hydrogels. Utrecht: VSP Publishers; 1995. p. 1081–2.
11. Dostinex. www.rxlist.com/cgi/generic/cabergoline.htm.
12. Eckelman WC. Radiolabeling with technetium-99m to study high-capacity and low- capacity biochemical systems. Eur J Nucl Med. 1995;22:249–63.
13. Freemark M, Fleenor D, Driscoll P, Binart N, Kelly PA. Body weight and fat deposition in prolactin receptor-deficient mice. Endocrinology. 2001;142(2):532–7.
14. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. Cell. 2004;116(2):337–50.
15. Garron JY, Moinereau M, Pasqualini R, Saccavini JC. Direct ^{99m}Tc labeling of monoclonal antibodies: radiolabeling and *in-vitro* stability. Int J Radiat Appl Instrum. 1991;18:695–703.
16. Greenman Y, Tordjman K, Stern N. Increased body weight associated with prolactin secreting pituitary adenomas:weight loss with normalization of prolactin levels. Clin Endocrinol. 1998;48 (5):547–53.
17. Hamann A, Matthaei S. Regulation of energy balance by leptin. Exp Clin Endocrinol Diabetes. 1996;104(4):293–300.
18. Hamann A, Matthaei S. The plasma leptin concentration is closely associated with the body fat mass in nondiabetic uremic patients. Am J Nephrol. 1999;19(4):485–91.
19. Karami K, Merclin N, Brouneus F, Beronius P. On iontophoretic delivery enhancement: ionization and transport properties of lidocaine hydrochloride in aqueous propylene glycol. Int J Pharm. 2000;201(1):121–4.
20. Korner J, Lo J, Freda PU, Wardlaw SL. Treatment with cabergoline is associated with weight loss in patients with hyperprolactinemia. Obes Res. 2003;11(2):311.
21. Leong KW, Bowman K. Chitosan nanoparticles for oral drug and gene delivery. Int J Nanomedicine. 2006;1(2):117–28.
22. Li L, Nandi I, Kim KH. Development of an ethyl laurate based microemulsion for rapid-onset intranasal delivery of diazepam. Int J Pharm. 2002;237:77–85.
23. Lisbeth I. Transport of drugs from the nasal cavity to central nervous system. Eur J Pharm Sci. 2000;11:1–18.
24. Lisbeth I. Nasal drug delivery: problems, possibilities and solutions. J Control Release. 2003;87:187–98.
25. Mercer JG, Archer ZA. Diet-induced obesity in the Sprague–Dawley rat:dietary manipulations and their effect on hypothalamic neuropeptide energy balance systems. Biochem Soc Trans. 2005;33 (5):1068–72.
26. Misra A, Ganesh S, Shahiwala A. Drug delivery to the central nervous system: a review. J Pharm Pharmaceut Sci. 2003;6: 252–73.
27. Misra A, Vyas TK, Shahiwala A, Marathe S. Intranasal drug delivery for brain targeting. Curr Drug Discov. 2005;2(2):165–75.
28. Peltola S, Saarinen-Savolainen P, Kiesvaara J, Suhonen TM, Urtti A. Microemulsions for topical delivery of estradiol. Int J Pharm. 2003;254(2):99–107.
29. Pattarini F, Gasco MR, Gallarate M. On the release of prednisone from oil in water microemulsions. Farmaco Prat. 1988;43(10):325–30.
30. Rolan I, Piel G, Delattre L, Evrard B. Systemic characterization of oil-in-water emulsions for formulation design. Int J Pharm. 2003;263:85–94.
31. Saha GB. Methods of radiolabeling. In: Saha GB, editor. Physics and radiobiology of nuclear medicine. New York, NY: Springer-Verlag; 1993. p. 100–6.
32. Sallee VL, Desantis L Jr, Mclaughlin MA, Maniar ML. Use of cabergoline and related ergoline derivatives for controlling intraocular pressure. United States Patent 5705510. 1998.
33. Sipos P, Horvat S, Feher A, Wolburg H, Veszelka S, Toth A, *et al*. Sodium hyaluronate as a mucoadhesive component in nasal formulation enhances delivery of molecules to brain tissue. Eur J Pharm Biopharm. 2009;72(1):252–9.
34. Songkro S, Junyaprasert VB, Boonme P, Krauel K, Rades T. Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o Brij 97-based microemulsions. J Pharm Pharmaceut Sci. 2007;10(3):288–98.
35. Thomas TC, Storlien LH, Huang XF, Lin S. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes. 2000;24(5):639–46.
36. Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol. 2001;53:3–21.
37. Vyas TK, Babbar AK, Sharma RK, Misra A. Preliminary brain-targeting studies on intranasal mucoadhesive microemulsions of sumatriptan. AAPS PharmSciTech. 2006;7:E1.
38. Willimann H, Walde P, Luisi PL, Gazzaniga A, Stropopolo F. Lecithin organogel as matrix for transdermal transport of drugs. J Pharm Sci. 1992;81(9):871–4.
39. Gao Y, Du G, Nie S, Pan W. The permeation of nalmefene hydrochloride across different regions of ovine nasal mucosa. Chem Pharm Bull. 2006;54(12):1722–4.
40. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, *et al*. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364:937–52.
41. Zhang Q, Jiang X, Xiang W, Lu W, Su L, Shi Z. Preparation of nimodipine- loaded microemulsion for intranasal delivery and evaluation of the targeting efficiency to brain. Int J Pharm. 2004;275:85–96.