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Optimization of brain targeted gallic acid nanoparticles for improved antianxiety-like activity

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ABSTRACT

Ligand coated nanoparticles may improve brain uptake of drugs. To formulate brain targeted nanoparticles of gallic acid (GA) for improving its antianxiety-like activity. The nanoparticles were prepared and optimized to minimize particle size and maximize percent drug entrapment efficiency using two factor three level (3^2) central composite design. Pure GA, optimized ligand coated nanoparticles of GA (cGANP) and corresponding uncoated nanoparticles (GANP) were administered to Swiss albino mice for seven consecutive days and evaluated in vivo for their antianxiety-like activity. Behavioral studies revealed that cGANP significantly improved antianxiety-like activity in mice. The plasma nitrite level decreased with GA, GANP and cGANP (most pronounced for cGANP) treated group as compared to saline treated control group while no change in plasma corticosterone levels was observed in any treatment. The treatments (except alprazolam) did not show any significant effect on locomotor activity of mice. The antianxiety-like activity may be attributed to decreased plasma nitrite level and effect was improved by enhanced brain uptake of GA via ligand coated nanoparticles. Thus antianxiety-like activity of GA was significantly improved formulating it as ligand coated nanoparticles. On the other hand, no significant difference was observed between antianxiety-like activity by administration of pure GA and GANP.

1. Introduction

Neuropsychiatric disorders are expected to rise sharply [1,2]. Anxiety disorders are the most common among the mental disorders, affecting nearly 1 in 5 adults in the U.S. alone [2,3]. Gallic acid is a natural anti-oxidant and may be utilized for its neuroprotectant activity [4]. It possesses significant anti-anxiety like effect at 20 mg/kg dose (*i.p.*) [5].

Drug delivery systems are developed to improve the efficacy of a therapeutically active compound and minimize its toxic side effects. This can be achieved by increasing the amount and duration of drug in the vicinity of the target cells and minimizing its exposure to non-target cells [4]. Blood brain barrier (BBB) is the major obstacle for delivery of drugs acting on central nervous system (CNS). One of the strategies to overcome BBB and enhance the brain uptake of drug is ligand coated nanoparticulate drug delivery [2,4,6,7]. Tween 80® is a non-ionic surfactant and has been successfully utilized for the targeted delivery of drugs to brain [4,6,7]. The targeting to brain was proposed to be mediated by either endocytotic uptake by the brain capillary endothelial cells (BCEC) or by transcytosis [7]. Therefore, Tween 80® was chosen for targeted delivery of gallic acid to brain in the present investigation. The administration of cGANP (equivalent to 10 mg/kg GA, *i.p.*) for 7 consecutive days significantly increased the antidepressant-like activity of GA in mice [4]. The present study was designed to improve the antianxiety-like effect of GA in mice through optimized ligand coated nanoparticles. The antianxiety-like activity was evaluated using elevated plus maze and light/dark box model. The biochemical parameters, i.e., plasma nitrite level and plasma corticosterone levels were also estimated.

2. Materials

Chitosan was generously gifted by Central Institute of Fisheries Technology, Kochi, India. Gallic acid monohydrate was procured from Hi-Media, Mumbai, India. Other chemicals used during the study were of suitable analytical grade and were used as received.

Swiss male albino mice (25–30 g; 3 months old) were obtained from Disease Free Small Animal House, LLRUVAS, Hisar (Haryana) India. Since female sex hormone estrogen has antianxiety-like effect, therefore female mice were excluded and only male mice were used for present investigation [5]. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Registration no. 0436). Efforts were made throughout to

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Table 1
Composition, particle size and DEE of various batches of chitosan–TPP nanoparticle formulations as per experimental design.

Formulation code	Trial no.	Coded factor levels		Particle size (nm)	DEE (%)
		X_1	X_2		
9	1	0	0	122.4	92.75
7	2	0	−1	321.1	94.21
12	3	0	0	105.7	92.53
2	4	1	−1	509	87.35
8	5	0	1	242.4	89.93
10	6	0	0	176.4	91.32
13	7	0	0	153.7	92.37
3	8	−1	1	208.4	95.67
11	9	0	0	78.82	94.37
4	10	1	1	467.1	82.15
6	11	1	0	436	83.97
1	12	−1	−1	232.2	94.32
5	13	−1	0	165.9	97.24
Translation of coded levels in actual units					
Coded level	−1	0	+1		
X_1: chitosan (%)	0.05	0.15	0.25		
X_2: Tween 80® (%)	1	1.5	2		

minimize animal discomfort and to use minimum number of animals ($n = 6$) for statistical significance. All animals were acclimatized to laboratory environment for seven days before testing.

3. Methods

3.1. Preparation and characterization of chitosan nanoparticles

3.1.1. Experimental design and preparation of chitosan nanoparticles

The chitosan NP were prepared by modified ionotropic gelation method [2,4]. Briefly, 0.1% w/v chitosan (CS) in 2% acetic acid solution was prepared and pH was adjusted to 5.6 by adding aqueous solution of sodium hydroxide or acetic acid solution. A constant amount of GA (50 mg) was added to CS solution followed by drop wise addition of an aqueous solution of TPP (chitosan:TPP::1:3) with constant stirring for 1 h at 1000 rpm using magnetic stirrer (GANP batch). For coating (cGANP batch), 1.5% Tween 80® (SD fine Chem. Ltd., Mumbai, India) was added after 1 h with continuous stirring at low speed (500 rpm). Thereafter, in order to separate nanoparticles, centrifugation of the dispersion was carried out at 15,000 rpm for 60 min at 10 °C which yielded nanoparticles containing pellet. To achieve the reproducible results, all the pellets were washed, re-dispersed in constant volume (20 mL) of HPLC grade water (Sisco Research Lab. Pvt. Ltd., Mumbai, India) and sonicated using probe sonicator for 2 min. Two milliliter of this suspension was further diluted 10 times, sonicated (2 min) and analyzed for particle size, size distribution and zeta potential. The undiluted nanoparticles were lyophilized using lyophilizer (Alpha 2–4 LD Plus CHRIST, Germany) after adding D-mannitol (Hi-Media Lab. Pvt. Ltd., Mumbai, India) as cryoprotectant in order to avoid particle agglomeration and adsorbed nanoparticles were obtained [4]. Limits and ranges for chitosan and Tween 80® for the optimization studies were set based on our previous findings. The optimization study was carried out using a two factor three level central composite design, CCD (with $\alpha = 1$). Three levels each of the two factors factor X_1 (i.e., percent CS concentration; w/v) and X_2 (i.e., percent Tween 80® concentration; w/v), were adopted for further investigations as required by the design, and the factor levels were suitably coded. Table 1 summarizes the 13 experimental runs studied employing different levels of the two factors.

3.1.2. Particle size and PDI

Particle size (hydrodynamic diameter) and PDI of the formulated NP were determined by dynamic light scattering technique using ZetaSizer Nano ZS90 (Malvern Instruments Limited, UK). All measurements were carried out after 10 times dilution of NPs with HPLC grade water. All measurements were carried out in triplicate and reported as mean ± SD.

3.1.3. Percent drug entrapment efficiency (%DEE)

The supernatant after centrifugation of NP (at 10,000 rpm) was collected, filtered through 0.45 μm filter, and amount of drug present was determined by UV spectrophotometer ($\lambda_{max} = 205$ nm). A standard calibration curve of concentration versus absorbance was plotted for this purpose. The regression equation is given by $y = 0.092x + 0.011$, with a correlation coefficient of $r^2 = 0.999$, where y represents absorbance (optical density) and x represents the concentration (in μg/mL). The amount of drug in supernatant (w) was then subtracted from the total amount of drug added (W, 100 mg in this case) and %DEE was calculated [4].

3.1.4. Optimization data analysis

Design Expert Software, DES ver.8.0.7.1 (Stat-Ease, Minneapolis, MN, USA) was employed to fit full second order polynomial equations with added interaction terms to correlate the studied responses with the examined variables. The response variables for systematic optimization were particles size and %DEE. The optimum formulations' prognosis was conducted by locating feasible space and secondly, an exhaustive grid search was conducted to obtain the possible solutions. The optimum solution was also located by the software using the overlay plot. The optimized Tween 80® coated batch (cGANP) and the corresponding uncoated batch was (GANP) were utilized for further in vitro and in vivo studies.

3.1.5. In vitro drug release

In vitro drug release from optimized batch of drug-loaded GANP and cGANP was evaluated using the equilibrium dialysis technique. Nanoparticles (equivalent to 1 mg GA) were suspended in 5 mL phosphate buffer saline (PBS) pH 7.4 and placed in a dialysis membrane bag (Himedia, MWCO, and molecular mass cut off 12,000–14,000, pore size 2.4 nm). The membrane bag was placed in 300 mL PBS (37 ± 1 °C). The rpm was set at 50 to avoid the excessive torque produced at higher rpm which may otherwise rupture the dialysis membrane. At regular time intervals, 5 mL aliquots were collected and equal volume of fresh PBS was replaced into release system. The supernatant was separated by centrifugation and evaluated for the concentration of GA using UV Visible Spectrophotometer.