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Research paper

Development of potent oral nanoparticulate formulation of coenzyme Q10 for treatment of hypertension: Can the simple nutritional supplements be used as first line therapeutic agents for prophylaxis/therapy? ☆

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Abstract

Coenzyme Q10 (CoQ10) is an antioxidant with well-established pharmacological activities against several chronic diseases; however, it is marketed only as a nutritional supplement without any claims of its therapeutic activity and one of the reasons for this could be the poor oral bioavailability rendering difficulties in administering this molecule to achieve therapeutic concentrations. Therefore, the present investigation was aimed at improving the oral bioavailability of CoQ10 by delivering it as nanoparticulate formulation. Biodegradable nanoparticulate formulations based on poly(lactide-co-gylcolide) (PLGA) were prepared by emulsion technique using quaternary ammonium salt didodecyldimethylammonium bromide (DMAB) as a stabilizer. The effect of initial CoQ10 loading on entrapment efficiency and the particle size was studied using 5–75% initial load resulting in good entrapment efficiency (61–83%) without any appreciable increase in the particle size for 5–30% loading (107–110 nm). However, 50% and 75% led to increase in particle size with no appreciable changes in entrapment efficiency. The intestinal uptake of CoQ10 as a suspension in carboxymethylcellulose (CMC), a commercial formulation and the developed nanoparticulate formulation was studied in male Sprague–Dawley (SD) rats and found to be 45%, 75% and 79%, respectively, suggesting that solubility and permeability related problems of CoQ10 were overcome by nanoparticulate formulation. Furthermore, the developed nanoparticulate formulation was evaluated for its therapeutic potential in renal hypertensive animals (Goldblatt 2K1C model), demonstrating improved efficacy at a 60% lowered dose as compared to CoQ10 suspension and superior efficacy than the commercial formulation at an equal dose. Together, these results indicate the potential of nanotechnology in improving the therapeutic value of molecules like CoQ10, facilitating its usage as first line therapeutic agent thus revolutionizing its role in current medical therapy.

Keywords: Antioxidants; Bioavailability; Biodegradable; Free radical; Hypertension; Nanoparticles; Oral delivery

1. Introduction

The discovery of the role of free radicals in cancer, cardiovascular diseases, diabetes, autoimmune diseases, neurodegenerative disorders and aging has opened up a new arena in health care which has resulted in an extensive search for antioxidants and their role as prophylactic and therapeutic agents. Various antioxidants starting from classical vitamins to polyphenols and carotenoids have been

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actively explored for their beneficial role in oxidative stress mediated consequences. Some of the widely investigated antioxidants are ascorbic acid, α-tocopherol, epigallocatechin-3-*O*-gallate (EGCG), lycopene, quercetin, genistein, ellagic acid, Coenzyme Q10 (ubiquinone) and indole-3-carbinol. Numerous animal studies as well as several clinical trials have demonstrated the ability of these compounds to prevent or cure one or more free radical mediated disorders. These antioxidants have also been actively explored and used for cosmetic applications such as anti-aging creams. In spite of proven pharmacological actions in humans, these antioxidants have failed to gain the status of main line drugs, and instead, are only being used as nutritional supplements for prophylaxis or adjuvant therapy. The failure of these agents to be recognized as first line agents for treatment of disease is due to their poor physicochemical and biopharmaceutical properties leading to their low oral bioavailability from conventional dosage forms. Apart from this, a lack of thorough investigation of novel approaches to facilitate the delivery of these molecules and the demanding regulatory procedures for their approval as therapeutic agents has also restricted their use [1].

One such potent but pharmaceutically challenging antioxidant is Coenzyme Q10 (CoQ10), a benzoquinone with 10 isoprenoid units. It functions as a coenzyme in the energy-producing metabolic pathways of every cell of the body. It acts as a medium for transfer of electrons from NADPH and succinate dehydrogenase to the cytochrome system, thus performing a vital function in ATP synthesis process. CoQ10 is found in the inner mitochondrial membrane where it acts as a cofactor for the mitochondrial enzymes (complexes I, II and III) that take part in oxidative phosphorylation. In addition to its role in the respiratory chain, CoQ10 functions as an antioxidant, scavenging free radicals and inhibiting lipid peroxidation [2,3]. Several studies have provided evidence of the potential of CoQ10 in prophylaxis and therapy of various disorders related to oxidative stress. CoQ10 has been found to be effective in cardiovascular disorders like cardiomyopathy, hypertension, angina pectoris and atherosclerosis [4,5]. It has also been found to be beneficial in cancer, neurodegenerative disorders, periodontal diseases and in cardiotoxicity caused by doxorubicin [6–8]. While there are numerous studies with human subjects on the therapeutic efficacy of CoQ10 supplementation for various indications, very little information is available regarding its bioavailability. Since CoQ10 is insoluble in water, the efficiency of absorption and bioavailability from foods and supplement is poor. Because of its poor aqueous solubility and high molecular weight (863 Da), CoQ10 has presented a challenge in the development of formulations for oral administration. Many different approaches for formulating CoQ10 have been reported; some of these have been introduced into the market as oil-based or powder-filled capsule formulations. However, the oral bioavailability of these formulations differs widely [9,10]. CoQ10 is a molecule with multifunctional activities, unlike the drugs in current use. The therapeutic benefits of this molecule cannot be overlooked and there is every need to apply novel drug delivery strategies to use it to its potential.

Recently, polymeric nanoparticles have been actively explored as oral delivery vehicles for pharmaceutically challenging molecules [11–13]. It has been shown that nanoparticles can improve the oral bioavailability of drugs that have poor water solubility and high molecular weight [14,15]. Following oral administration, particles less than 500 nm cross the M cells in the Peyer's patches and the mesentery on the surface of gastrointestinal mucosa, delivering the drug to the systemic circulation [16,17]. Although polylactide-co-gylcolide (PLGA), a polyester, has been extensively used for micro- and nanoparticulate formulations, not much has been reported on its use in oral delivery. Similarly not much work has been done demonstrating the efficacy of the nanoparticulate system in diseased models. Therefore, efforts were expended to investigate the potential of PLGA nanoparticles for oral delivery of CoQ10 by demonstrating its efficacy in a disease model. This study establishes the suitability of polymeric nanoparticles in delivering CoQ10 through the oral route, and initiates a paradigm shift in health care by establishing the efficacy of a nutritional supplement as first line therapeutic agent.

2. Materials and methods

2.1. Materials

CoQ10 was a free gift provided by Tischon Corp. (Westbury, NY). PLGA 50:50 (intrinsic viscosity 0.41 dL/g in chloroform at 25 °C) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Didodecyldimethylammonim bromide (DMAB) and hexadecyltrimethyl ammonium bromide (HTAB) were purchased from Sigma (USA). HPLC grade ethanol and methanol were purchased from Les Alcools De Commerce Inc. (Ontario) and J.T. Baker (USA), respectively. All other chemicals used were of analytical grade. Ultrapure water (SG Water Purification Systems, Barsbuettel, Germany) was used for all experiments.

2.2. Method for nanoparticle preparation

Nanoparticles were prepared by emulsion–diffusion–evaporation technique with slight modification from the reported method [18]. In brief the method used was as follows: 50 mg of PLGA (50:50) was dissolved in 2.5 ml of ethyl acetate at room temperature and stirred for 2 h. The organic phase was then emulsified with 5 ml of an aqueous phase containing stabilizer (1% w/v DMAB). The resulting o/w emulsion was stirred at room temperature for 1 h before homogenizing at 15,000 rpm for 5 min using a high-speed homogenizer (Polytron PT4000; Polytron Kinematica, Lucerne, Switzerland). To this emulsion, water was added with constant stirring which resulted in nanoprecipitation. CoQ10 loaded nanoparticles were pre-

pared by dissolving CoQ10 in minimum volume of ethyl acetate and adding to polymeric solution following the same method.

2.3. Particle size and zeta measurements

The size of nanoparticles was determined by dynamic light scattering (Nano ZS, Malvern Instruments, Malvern, UK), taking the average of five measurements. The polydispersity index (PDI) which is a dimensionless number indicating the width of the size distribution, having a value between 0 and 1 (being 0 for monodisperse particles), was also obtained. The surface charge of nanoparticles, characterized by the zeta potential, was determined by measuring electrophoretic mobility under an electric field, as an average of 30 measurements.

2.4. Entrapment efficiency

Entrapment efficiency is the percentage of ratio of drug initially taken for loading to that actually incorporated into the nanoparticles. It was determined by centrifuging the drug loaded nanoparticles and separating the supernatant. The supernatant was analyzed for free drug using HPLC and entrapment efficiency was determined.

2.5. Analysis of CoQ10

The drug was analyzed using Waters high-performance liquid chromatography (HPLC) system consisting of Millennium 32 acquisition software, 515 pumps, 717plus Autosampler, 2487 Dual Absorbance Detector (Waters Corp., Ireland). Separation was achived using a reversed phase C18 Nova Pack® (25 cm × 4.6 mm, 5 μm. Waters, USA) column fitted with Nucleosil, C18 (Macherey-Nagel, Germany) guard column. A mixture of 9:1 of ethanol and methanol was used as the mobile phase at a flow rate of 1 ml/min with UV detection at 275 nm.

2.6. In vitro drug release studies

The release of CoQ10 from the nanoparticles was determined by dialysis membrane method. To study the effect of initial drug loading on *in vitro* release profile, 20% and 50% (w/w of polymer) initial CoQ10 loaded nanoparticles were selected. PLGA nanoparticles (corresponding to 1 mg of CoQ10 entrapped) were redispersed in 1 ml of 1% Tween 20 solution in dialysis bags (Sigma) with a molecular mass cut-off of 12,000 Da. The bags were suspended in 10 ml of 1% Tween 20 solution at 37 °C in a shaking water bath (LabTech, Daihan LabTech Co. Ltd., Korea) at 50 rpm. At predetermined intervals, the release medium was completely replaced with 1% Tween solution at 6 and 12 h on first day followed by sampling at 24 h interval for the first 5 days and then 48 h sampling for the remaining days. The amount of CoQ10 released in the medium was estimated by HPLC. *In vitro* release was also performed in 1% HTAB solution in a very similar method as described above.

2.7. In situ uptake studies

Closed loop method was used to assess the absorption characteristics of CoQ10 as a suspension in carboxymethylcelulose (CMC), CoQ10 loaded nanoparticles and the commercial formulation. Overnight fasted male SD rats were anesthetized by intraperitoneal administration of thiopentone (50 mg/kg). The intestine was exposed through a midline incision and a closed loop of 10 cm length was prepared on the upper jejunum by ligation at both ends. The aqueous drug suspension, drug loaded nanoparticles and commercial formulation were suitably diluted/constituted as to contain 20 mg/ml of CoQ10 and 1 ml of these preparations was injected into the loop with a syringe. The entire intestine was restored to the abdominal cavity and body temperature was maintained during anesthesia. After 2 h of study period, the loops were rapidly isolated from the body and the contents of the loop were recovered. The lumen of the intestine was rinsed with 10 ml water followed by 10 ml of methanol. The water and methanol solutions were collected and processed. Appropriate dilutions were made for quantifying the unabsorbed drug remaining in the solution by HPLC.

2.8. Efficacy study in hypertensive animals

Goldblatt method 2-kidney 1-clip (2K1C) was used for induction of hypertension [19,20]. The animals were divided into five groups. Group 1 was sham operated; Group 2 was treated with vehicle; Group 3 received seven doses of CoQ10 suspension (100 mg/kg); Group 4 received three doses of commercial formulation (100 mg/kg) while Group 5 received three doses of CoQ10 (100 mg/kg) in the form of prepared nanoparticulate formulation after surgery. The study design to evaluate the efficacy of nanoparticles in hypertensive rats is briefed in Table 1.

All the animal experiments were performed according to a protocol duly approved by the Institutional Animal Ethics Committee (IAEC) of NIPER. Male SD rats weighing around 200 g were acclimatized to the environment one week prior to the experimentation. On the day of experimentation, the animals were anesthetized using ketamine (40 mg/kg i.p.) and xylazine (2 mg/kg i.p.). Abdominal area was thoroughly shaved and sterilized with liberal application of 70% alcohol. Midline incision was made on the upper ventral abdomen to expose the left kidney. Renal vessels including artery and vein were delicately picked up and renal artery was separated from the vein using bent tissue forceps. Renal artery was meticulously clamped in the middle or close to the kidney using silver strips (approx. 0.80–1.00 cm length, 0.3 mm width and 0.022 mm thick) in such a way that it partially blocked the blood supply to the kidney. Muscle was then sutured using chromic catgut (4/0) and skin was properly opposed

Table 1
Study design to evaluate efficacy of nanoparticles in hypertensive rats

Groups	Day after surgery									
	SURGERY	1	2	3	4	5	6	7	12	15
Sham Operated (no clipping)		----------							BP recording	BP Recording and Lipid peroxidation measurements in plasma
Vehicle Treated		V	V	V	V	V	V	V		
Suspension (100 mg/kg)		D	D	D	D	D	D	D		
Commercial Formulation (100 mg/Kg)		D			D			D		
*Nanoparticles (100 mg/kg)		D			D			D		

V and **D** indicate the day on which the vehicle and drug have been administered to the rats after surgery, respectively. *CoQ10 is more efficacious as nanoparticulate formulation at 60% lower dose than suspension form and at equal dose in comparison to marketed formulation.

using skin clips. Animals were subsequently allowed to recover. The animals were treated with gentamicin (2 mg/kg i.p.) before and 2 days after surgery to prevent infection. Sham treated rats were kept as normal controls.

2.9. Blood pressure recording

Blood pressure (systolic and diastolic) was recorded on 12th and 15th day after surgery using tail cuff blood pressure recorder (IITC Inc., Life Science Instruments, Model No. 29,229; California, USA) [21]. Rats were pre-warmed in the detector chamber (27–30 °C) for 20 min before the blood pressure recording. Three recordings were taken for each rat and the mean was calculated.

2.10. Lipid peroxidation measurements

Malondialdehyde (MDA) levels, an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid (TBA) to produce a red complex that has a peak absorbance at 532 nm. On the 15th day after surgery, blood was collected from tail vein of rat under mild ether anesthesia into centrifuge tubes containing heparin (less than 5 I.U.). Blood samples were centrifuged at 5000 rpm for 5 min to separate plasma which was subsequently used for the estimation of lipid peroxidation. The procedure for estimation in brief is as follows: 0.1 ml of plasma was taken in a test tube and mixed with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% glacial acetic acid and 1.5 ml of 0.8% TBA. Following these additions, tubes were mixed and heated at 95 °C for 1 h on a water bath. After heating, the samples were cooled and 1 ml of water was added. The absorbance of this solution was measured at 532 nm wavelength and is proportional to the amount of MDA in the blood [22]. A calibration curve was prepared using different concentrations of MDA.

3. Results and discussion

Nanoparticles were prepared by adopting emulsion-diffusion-evaporation technique. The series of studies carried out in our laboratory indicated the potential of DMAB as a stabilizer for the preparation of nanoparticles intended for oral administration [18,23,24]. The advantage of DMAB stabilized particles in oral delivery could be due to its cationic property and its ability to produce smaller sized particles irrespective of polymer molecular weight or copolymer ratio. The blank nanoparticles were 100.9 ± 2.8 nm in size with PDI of 0.095 ± 0.007.

With drug loading, an increase in size of particles by few nanometers was observed, however this increase was not proportional with the increase in initial load. Effect of initial drug loading on particle characteristics was investigated using initial load of 5–75% w/w with reference to the polymer weight. No significant change was observed in the particle size, particle size distribution and zeta potential with increase in drug loading from 5% to 30%; however, a slight increase in the particle size and its distribution was observed with 50% and 75% loading. A similar trend was observed in terms of entrapment efficiency (Table 2). The broad range of initial load (5–75%) ratio of CoQ10 to the polymer in the present study suggests a commercially feasible product profile that can lead to different dose formulations for a wide range of applications.

The prepared nanoparticles were further evaluated for *in vitro* drug release. An appropriate design to conduct this

Table 2
Effect of initial drug loading on particle characteristics and entrapment efficiency

Initial drug loading	Size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
5	107.3 ± 2.2	0.10 ± 0.02	81.9 ± 2.1	61.7 ± 3.9
10	110.3 ± 1.2	0.11 ± 0.01	83.3 ± 3.9	74.3 ± 3.0
15	108.0 ± 3.6	0.10 ± 0.01	81.7 ± 2.1	79.2 ± 2.6
20	111.7 ± 6.4	0.10 ± 0.04	81.6 ± 1.3	82.0 ± 3.3
30	110.3 ± 3.0	0.11 ± 0.02	81.3 ± 2.8	79.4 ± 0.8
50	125.8 ± 4.3	0.13 ± 0.03	80.5 ± 2.5	80.0 ± 2.8
75	131.6 ± 2.8	0.14 ± 0.03	80.7 ± 2.9	83.1 ± 1.2

The zeta potential values reported are in the pH range 4.00–4.85. The values reported are means ± SD ($n = 3$).

study was difficult to work out due to a number of technical problems, the foremost being creating sink conditions for highly lipophilic CoQ10. For poorly soluble CoQ10, adequate release medium providing sink conditions was not obtained with aqueous solutions within physiological pH. For this reason, an aqueous solution containing a surfactant was used to enhance its solubility. Commonly acceptable ionic and nonionic surfactants include sodium lauryl sulphate (SLS), Tweens, hexadecyltrimethylammonium bromide (HTAB), polyethylene glycol tert-octylphenyl ether (Triton) cremophore, cyclodextrins and lecithin. Nonionic surfactant Tween being considered more biologically relevant [25] was our first choice as surfactant and *in vitro* release was first carried out in 1% Tween 20 solution.

In 1% Tween 20 solution, the 20% CoQ10 loaded nanoparticles showed no release at all. The 50% CoQ10 loaded nanoparticles showed an initial burst effect, which continued for 48 h and resulted in around 1.7% cumulative release (Fig. 1). However, after 48 h there was no observable release from the nanoparticles. The release studies were continued for 12 days. At the end of 12 days since no release was being observed in both the batches, the amount of CoQ10 remaining in the particles was estimated after its extraction from the nanoparticles, followed by HPLC analysis. The amount of CoQ10 remaining was 85% and 83% for 20% and 50% loaded nanoparticles, respectively (Fig. 2). The amount remaining demonstrated that CoQ10 did not undergo any major degradation. Failure of nanoparticles to release CoQ10 may be due to possible interaction or complexation between the polyethylene groups of Tween 20 with isoprene groups of CoQ10. There is further possibility of the 10 isoprene units of CoQ10 preventing Tween 20 solution to penetrate inside the polymeric matrices hence preventing the hydrolysis of ester bonds and drug release.

The subsequent release studies were carried out in 1% HTAB solution [25], where a burst effect was observed for both 20% and 50% loaded nanoparticles. The release from 20% loaded nanoparticles was slow and about 4% of drug was released in 21 days, on the other hand 10% drug release was observed for particles with 50% loading over 21 days (Fig. 1). This faster release observed with 50% loaded particles in comparison to 20% loaded particles can be attributed to the difference in the proportion of polymer to CoQ10 entrapped. The entrapment efficiency being very similar for both the drug loaded batches, 20% loaded nanoparticles had higher ratio of polymer to CoQ10 than the 50% loaded nanoparticles resulting in a slower release. As the release was found to be very slow,

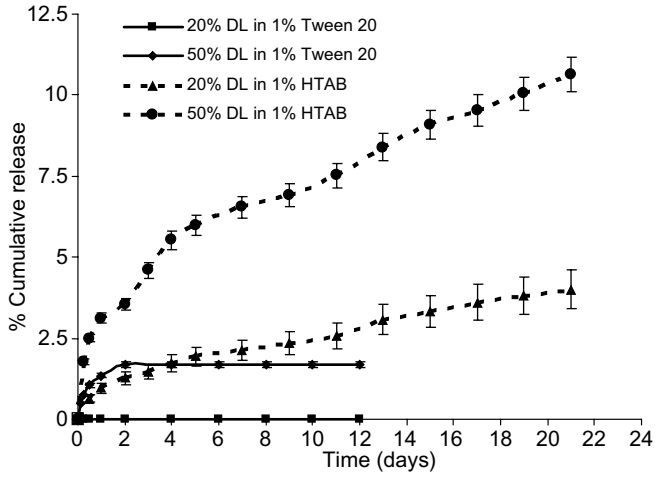

Fig. 1. *In vitro* release profile of 20% and 50% CoQ10 loaded nanoparticles in 1% Tween 20 and 1% HTAB solution as dissolution media (the values reported are means ± SD ($n = 3$), DL-drug loading).

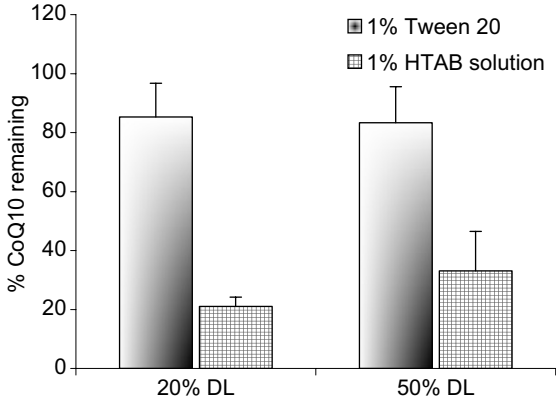

Fig. 2. Percent of CoQ10 remaining in the nanoparticles after release studies in 1% Tween 20 and 1% HTAB as dissolution media, respectively (the values reported are means ± SD ($n = 3$), DL-drug loading). The amount of CoQ10 remaining was 85% and 83% for 20% and 50% CoQ10 loaded nanoparticles after 12 days of release in 1% Tween 20 and 20% and 33% after 21 days of release in 1% HTAB for 20% and 50% CoQl0 loaded nanoparticles, respectively.

the study was stopped at the end of 21 days and the amount of CoQ10 remaining in the particles was estimated as above. The amount of CoQ10 remaining was 20% and 33% for 20% and 50% drug loaded nanoparticles, respectively (Fig. 2). These figures clearly suggest the possible degradation of CoQ10 in 1% HTAB solution, which was well supported by degradation profile of CoQ10 in the same. From the profile it was observed that 50% of CoQ10 had degraded by 3rd day and 85% by 8th day of the initial amount (Fig. 3). However, more than 20% of CoQ10 in the nanoparticulate systems had remained even after 21 days in 1% HTAB solution. This demonstrated the protective effect of the nanoparticulate system. A detailed investigation needs to be carried out to determine a suitable *in vitro* release medium for CoQ10 nanoparticulate system. The factors that need to be considered are solubility and stability of CoQ10 in the medium, analytical limitations and compatibility of medium with the drug and dialysis membrane. An alternative approach would be to remove small amounts of the particles to find out the amount of drug remaining inside the particles. There are some practical challenges to it, like opening of the dialysis bags for sampling. Nevertheless, for drugs unstable in the release medium, this is a useful way of assessing release when the drug is protected in the delivery system, and the actual amount present in the release medium might not be indicative of the amount released since the last sampling.

In situ uptake studies are routinely conducted to study absorption characteristics of particles from the intestine [18,23]. The biggest advantage of the *in situ* system compared to the *in vitro* techniques is the presence of an intact blood and nerve supply in the experimental animals. Closed loop method which is a modification of perfusion technique was used to assess the absorption of CoQ10 and its formulations. Absorption assessed was based on the disappearance of nanoparticles/drug from the intestinal lumen, calculated on the basis of the drug remaining. Despite its advantages, this method is slightly limited because it relies on the disappearance of the drug from the luminal side as an indication of absorption, but does not always represent the rate of absorption of the drug into the systemic circulation. Also, not all the particles that failed to show up in the washing might have been taken up into the lymphatics or systemic circulation. CoQ10 in the form of CMC suspension, commercial formulation and nanoparticles was evaluated for *in situ* uptake in jejunum of SD rats. The uptake of CoQ10 in the form of suspension was found to be 45%, indicating the permeability related problems with the molecule. The uptake of commercial formulation and CoQ10 loaded nanoparticles was found to be 75% and 79%, respectively (Fig. 4). The crucial activity of any formulation administered orally depends on its ability to cross the biological barriers. The uptake studies have clearly demonstrated the ability of the nanoparticles to cross biological barriers and proved to be far more superior to the simple CoQ10 suspension and slightly better than the commercial formulation. However, the overall performance of the formulations depends on various other parameters like its ability to release the drug and circulate in blood; subsequently these nanoparticles were evaluated in renal hypertensive rats (Goldblat 2K1C model) to determine their *in vivo* performance and efficacy.

Clinical and experimental studies clearly indicate a deficiency of CoQ10 in hypertension [4]. Several *in vitro* and *in vivo* studies have demonstrated the likely benefits of CoQ10 in treatment of hypertension. Administration of CoQ10 significantly lowered blood pressure in animal models of hypertension such as spontaneously hypertensive rats (SHR), uninephrectomized rats treated with saline and deoxycorticosterone (DOCA) [26,27]. Many clinical trials have also demonstrated the potential of CoQ10 in treatment of hypertension in humans [28,29]. The beneficial effect of CoQ10 in hypertensive state is associated with a decrease in total peripheral resistance which is due to its direct action on the vascular wall. CoQ10 also acts as an antagonist for vascular superoxides, either scavenging them or suppressing their synthesis [30].

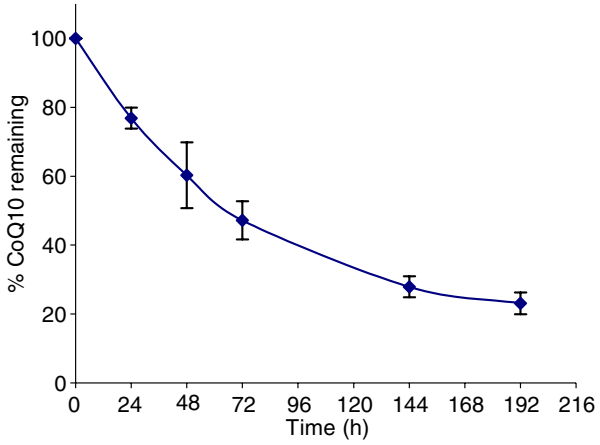

Fig. 3. Degradation of CoQ10 in 1% HTAB solution (the values reported are means ± SD ($n = 3$)). Fifty percent of the initial amount of CoQ10 was degraded by 3rd day and 85% by 8th day.

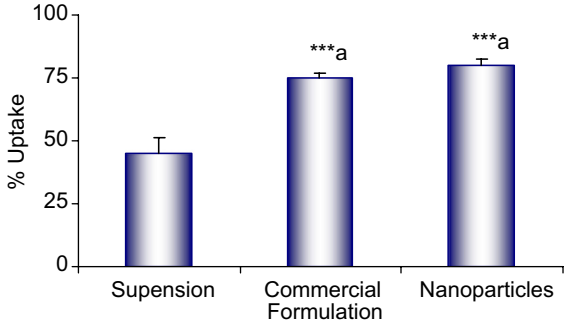

Fig. 4. *In situ* uptake of CoQ10 and its formulations (the values reported are means ± SD ($n = 3$)). Statistical analysis was performed by one way ANOVA followed by Tukey's test for multiple comparisons. $^{***}p < 0.001$; a vs suspension.

Goldblatt 2-kidney 1-clip (2K1C) model was used to assess the potency of different CoQ10 formulations which included CoQ10 suspension, commercial formulation and the prepared nanoparticles, with their treatment schedules highlighted in Table 1. The 2K1C model was initially optimized for evaluation of CoQ10's antihypertensive effect. Blood pressure (BP) was recorded before administration of CoQ10 and comparisons between pre-CoQ10 (i.e., CoQ10 given before the renal clipping surgery) and post-CoQ10 (i.e., CoQ10 given after renal clipping surgery) were conducted. It was observed the pre-CoQ10 was not effective in treatment of hypertension while post-CoQ10 was effective. CoQ10, being atypical antihypertensive drug, required few days to show its pharmacological action. CoQ10 was significantly effective in reducing the blood pressure from 12th day onwards after clipping (data not shown), as a result of which 12th and 15th day were fixed for BP recordings to evaluate different CoQ10 formulations. The model involves clipping of left renal artery which causes vasoconstriction of the artery, leading to increased renin synthesis and secretion from the clipped kidney. The renin released causes conversion of angiotensinogen to angiotensin I which is further converted to angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II is potent vasoconstrictor and it also acts on adrenal cortex to secrete aldosterone which causes salt and water retention leading to elevated BP. Free radicals are also produced which further mediate the physiological role of angiotensin II, resulting into augmentation in BP. The CoQ10 will scavenge the free radicals produced and prevent the augmentation of BP. It would also relax the vascular smooth muscles by its direct action and negotiate the angiotensin's vasoconstrictory action leading to reduction in BP and treatment of hypertension.

The vehicle treated group showed elevated blood pressure (systolic (*S*) 163.8 ± 2.8 and diastolic (*D*) 100.6 ± 1.3 mm Hg) as compared to sham operated (*S*: 125.2 ± 2.6 and *D*: 82.5 ± 0.1 mm Hg) on day 12 indicating the induction of hypertension due to renal clipping. CoQ10 at a dose of 100 mg/kg/daily administered as suspension in CMC (vehicle) was not able to bring the BP to normal values (*D*: 140.2 ± 3.7 and *S*: 89.7 ± 1.9 mm Hg) (Fig. 5), which could be due to poor oral bioavailability of CoQ10 when administered as conventional formulation. The poor oral bioavailability could be due to poor solubility and poor permeability, demonstrated by *in situ* studies (Fig. 4). The commercial formulation of CoQ10 at a dose of 100 mg/kg/once in 3 days also failed to bring the blood pressure to normal levels (*S*: 150.9 ± 2.5 and *D*: 93.1 ± 1.5 mm Hg) (Fig. 5), however from the *in situ* studies, it appears that permeability was not a problem with this formulation and was comparable to nanoparticles (Fig. 4). The failure of this formulation could be due to its inability to sustain the release of CoQ10 over extended period of time. Nanoparticulate formulations of CoQ10 at a dose of 100 mg/kg/once in 3 days (at 60% lower dose compared to suspension) succeeded effectively in bringing the blood pressure to normal (*S*: 125.9 ± 5.3 and *D*: 83.4 ± 2.4 mm Hg) (Fig. 5). Polymeric nanoparticles due to their ability to sustain the release of encapsulated agents and circulate in the system for extended periods of time made them much more effective even at 60% lower dose, supporting the improved bioavailability and efficacy. Overall, the effect of various CoQ10 treatments on hypertension (decrease in mm Hg) in comparison to the vehicle treated groups is as follows: nanoparticles (*S*: 38; *D*: 17), commercial formulation (*S*: 13; *D*: 8) and as suspension in CMC (*S*: 24; *D*: 11) suggesting a significant improvement in the performance of CoQ10 when administered as nanoparticulate formulation.

Similar results were obtained on 15th day after surgery. Systolic BP were found to be 141.2 ± 1.6, 149.8 ± 5.1 and 134.3 ± 4.1 mm Hg for suspension, commercial formulation and nanoparticle treated groups and diastolic BP were found to be 90.8 ± 0.7, 92.9 ± 4.9 and 85.1 ± 1.4 mm Hg, respectively (Fig. 6). The efficacy of three doses of CoQ10 nanoparticles in treating hypertension was again far more superior to seven doses of CoQ10 in suspension form. Three doses of nanoparticles were able to reduce the blood pressure by 30 and 15 mm Hg for systolic and diastolic BP

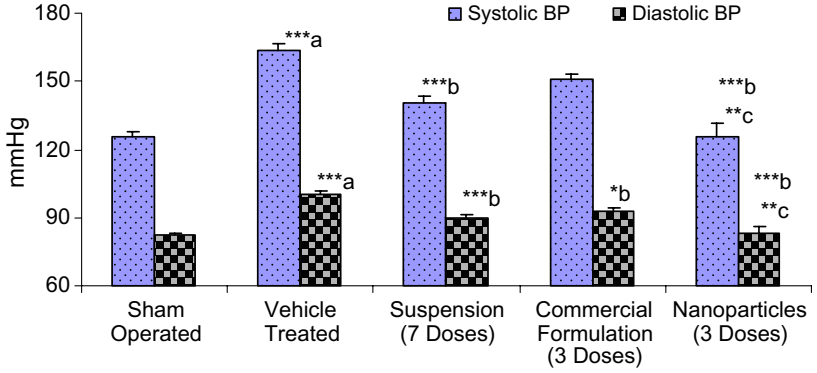

Fig. 5. Effect of different CoQ10 formulations on systolic and diastolic blood pressure on 12th day after surgery in Goldblatt hypertensive rats. Data represented as means ± SEM ($n = 5$–7). Statistical analysis was performed by one way ANOVA followed by Tukey's test for multiple comparisons. $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$; a vs sham operated group; b vs vehicle treated group; c vs commercial formulation.

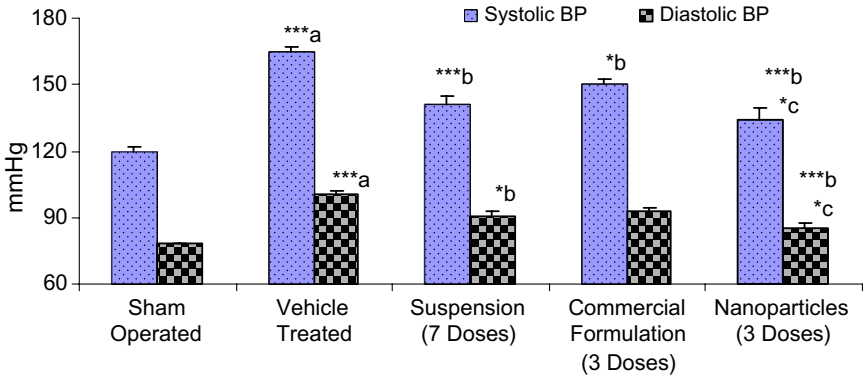

Fig. 6. Effect of different CoQ10 formulations on systolic and diastolic blood pressure on 15th day after surgery in Goldblatt hypertensive rats. Data represented as means ± SEM ($n = 5$–7). Statistical analysis was performed by one way ANOVA followed by Tukey's test for multiple comparisons. $^{***}p < 0.001$, $^{*}p < 0.05$; a vs sham operated group; b vs vehicle treated group; c vs commercial formulation.

in comparison to vehicle treated group (*S*: 163.9 ± 2.8 and *D*: 100.6 ± 1.3 mm Hg) while seven doses of suspension were able to reduce by 23 and 10 mm Hg, respectively. When compared to commercial product the nanoparticles were found to be more potent. The commercial formulation again reduced the BP marginally, by 15 and 7 mm Hg for systolic and diastolic, respectively. As observed from the data, CoQ10 nanoparticles were 50% more efficacious than the suspension form in treating hypertension at 60% lower dose and 85% more efficacious than the commercial formulation at equal dose. The improved efficacy clearly indicates the ability of nanoparticles to sustain the drug release and extend the circulation time, improving overall bioavailability.

Angiotensin II, which is produced as a result of renal clipping, increases the vascular superoxide anion and hydrogen peroxide (both are free radicals) production by activation of membrane bound NAD(P)H oxidase. These act on intracellular growth related proteins and enzymes. Increased vascular superoxide production also leads to reduced bioavailable nitric oxide (NO) and impairment of the endothelium-dependent relaxation, a characteristic state observed in hypertension. Along with the NO imbalance and endothelium impairment, the free radicals produced also cause lipid peroxidation. To investigate the role of free radicals in renal hypertension and to observe the free radical scavenging effect of CoQ10 and effect of various formulations on its efficacy, lipid peroxidation measurements were carried out by quantifying MDA levels. From the results it was very clear that renal clipping was able to increase the MDA levels in the plasma while, administration of CoQ10 decreased the MDA levels in treatment groups in comparison to vehicle treated group (Fig. 7). These clearly indicated the possible role of free radicals in hypertension. CoQ10 when administered as nanoparticles, was as effective as simple suspension even at a 60% reduced dose in reducing MDA levels. Interestingly the commercial formulation behaved similar to the nanoparticulate system though failed to reduce blood pressure, which needs to be understood. The pharmacodynamic efficacy, the ultimate evaluation parameter for any novel formulation, confirms formulation's ability to achieve desired therapeutic blood levels and its capability to circulate and release the drug at desired rate *in vivo*. The renal hypertension study precisely demonstrates the pharmacodynamic efficacy of CoQ10 nanoparticles in treatment of hypertension.

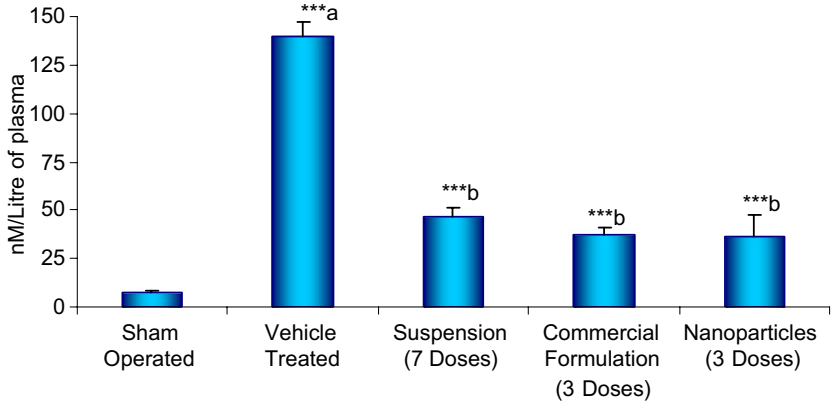

Fig. 7. Effect of different CoQ10 formulations on lipid peroxidation in hypertensive rats. Data represented as means ± SEM ($n = 3$). Statistical analysis was performed by one way ANOVA followed by Tukey's test for multiple comparisons. $^{***}p < 0.001$. a vs sham operated group; b vs vehicle treated group.

4. Conclusion

The present investigation illustrates that routinely used nutritional supplements such as CoQ10, which are generally found to be safe but associated with biopharmaceutical hurdles, can be used as first line therapeutic agents for prophylaxis and therapy by overcoming the problems associated with its delivery. CoQ10 loaded PLGA nanoparticles stabilized with DMAB administered orally have been found to be suitable for treatment of hypertension. The investigation also demonstrates the potential of polymeric nanoparticles for oral delivery of pharmaceutically challenging molecules.

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