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Enhanced oral bioavailability of Coenzyme Q10 (Ubiquinone) formulated in Ultrasome ™ drug delivery system.

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Abstract

Coenzyme Q10 (CoQ10, ubiquinone), a fat- soluble natural antioxidant with potential use as adjuvant therapy in heart diseases and to protect neurons against age-related degeneration, is a very lipophilic compound and poorly absorbed in the gut. CoQ10 was formulated using the Ultrasome™ proprietary drug delivery technology. The Ultrasome™ encapsulated CoQ10 showed high drug-tapping efficacy, better *in vitro* drug release and enhanced oral bioavailability compared to generic CoQ10. The positive effect of Ultrasome-CoQ10™ was observed among Israeli athletes with respect to muscle pain and fatigue after physical activity. Additionally, in several clinical trials and scientific research Ultrasome-CoQ10™ played a role in quality of life in patients with end-stage heart failure awaiting cardiac transplantation, in healing process of chronic skin lesions, in rehabilitation outcome following surgical repair of hip fracture and in protecting against 6-hydroxydopamine induced nigra lesions in rats which indicates is potential therapy for Parkinson's disease (PD) and other neurodegenerative diseases without side effects.

1. Introduction

Coenzyme Q10 (CoQ10) is a compound found naturally in virtually every cell in the human body with highest concentrations in the heart, liver , kidney and pancreas (1). Because of its ubiquitous presence in nature and its quinone structure (similar to that of vitamin K), CoQ10 is also known as ubiquinone (1). In 1957, Crane *et al*. (2) demonstrated that CoQ10 plays an important role as an electron carrier in motochonderial oxidative phosphorilation. Due to its involvement in ATP synthesis, CoQ10 affects the function of all cells in the body, making it essential for proper function of all tissues and organs. In addition, CoQ10 appears to increase adenosine triphosphate levels by preventing the loss of the adenine nucleotide pool from cardiac cells (3). CoQ10 also functions as a potent intercellular antioxidant and has

cell membrane stabilizing properties as well (4). Coenzyme Q10 has wide-ranging cellular properties that implicate it for the potential treatment of numerous diseases that may improve with mitochondrial antioxidant support. Numerous studies have investigated the benefit of CoQ10 supplementation for improving cardiovascular function via enhanced energy production, improved contractility of cardiac muscle, and its potent antioxidant activity- particularly prevention of LDL oxidation (*5,6*,7,8). Several trials demonstrated a mean decrease in systolic and diastolic blood pressure (9).

The effects of CoQ10 were investigated in neurologic indications such as Parkinson's Alzheimer's and Huntington's diseases (10, 11), mitochondrial abnormalities including the mitochondrial encephalomyopathis, stroke-like episodes (MELAS) syndrome, myoclonus epilepsy (12,13), and diabetes as Coenzyme Q10 has been considered for improving glycemic control through various mechanisms, including a decrease in oxidation stress (14, 15). CoQ10 was shown to inhibit the acute toxicity of the chemotherapy agent doxorubicin due to its antioxidant properties and the inhibition doxorubicin activity on CoQ10 dependent enzymes in cardiac and other tissues (16). Numerous studies have investigated the positive effect of CoQ10 supplementation for improving muscular injury and oxidative stress during exercise training (17), exercise performance (18) and physical fatigue sensation (19).

The structure of CoQ10 consists of a quinone ring attached to variable terpenoid side chains containing one to ten monounsaturated trans-iso prenoid units. The side chain is highly fat-soluble, which allows CoQ10 to be assimilated inside cells and is practically insoluble in water (20). The oral bioavailability of CoQ10 is generally very low and was found to be related to the dissolution rate of the formulation. Emulsions and microemulsions have been shown to be advantageous as vehicles for the oral delivery of lipophilic drugs resulting in improved oral bioavailability of water-insoluble compounds (21, 22). The objective of the present work was to examine the oral bioavailability of CoQ10 formulated using the Ultrasome^{TM} technology.

2. Materials and methods

2.1. Materials

Coenzyme Q10 was obtained from Global Marketing Associates, Inc. (San Franciso, CA). D-α-tocopherol was purchased from Merck (Germany). Lecithin was from Lipoid KG (Germany). Solid triglycerides were obtained from Hulls (Germany).

2.2. Methods

2.2.1. Formulation of CoQ10 in Ultrasomes

CoQ10 was dissolved together with the lipid ingredients (solid triglyceride, tocopherol succinate and phospholipids) in ethanol. The solvent was evaporated until complete dryness, and the dry drug lipid mixture was then hydrated with the aqueous phase by mechanical shaking. The resultant lipid dispersion was consequently homogenized by high pressure homogenizer (800 bar) to reduce the particle size to the submicron range. The solvents (EtOH+water) were evaporated by spray drying to final Ultrasome^{TM}-CoQ10 dry powder.

2.2.2. In vitro release of CoQ10

In vitro release of CoQ10 from Ultrasome^{TM}-CoQ10 formulation and commercial product (generic CoQ10) containing equivalent amounts of CoQ10 (50 mg) were determined by USP apparatus type 2 (paddles) contains 8 vassels, 75 rpm, filters (70 µM) In 750 ml of simulated gastric fluid (150 mM Nacl, pH=1.2, 37^{o}C) containing 0.1% Tween 80 as sink for 2 hr.

2.2.3. Determination of CoQ10

CoQ10 was determined in the commercial product, Ultrasome^{TM} formulation by extraction with Dole reagent (isopropanol:heptane:water, 45:36:17) and measuring absorbance at 270 nm using calibration curve. 0.5 ml of CoQ10 samples were added to 3.5 ml of Dole reagent and mixed thoroughly and the two phases were allowed to separate for 10 min at room temperature. CoQ10 was extracted selectively in Dole heptanes upper phase which was transferred to a quartz cuvette for absorbance measurements.

2.2.4. Human oral Bioavailability

2.2.4.1. Patients

The selected population was hospitalized, geriatric patients, undergoing continuous treatment with various medications. Patients were randomly assigned into two groups. Each subjects received a single dose of 90 mg of CoQ10 in hard gelatin capsules of either Ultrasome-CoQ10^{TM} or generic CoQ10 (control group). The inter

subject base line variability was minimized by selection the individuals of those with prestudy endogenous CoQ10 plasma levels of 0.30 CoQ10 µL/mL. Ten to twelve participants in each group fulfilling these criteria were enrolled in the study and compared for the effect of supplemented CoQ10 formulation on mean CoQ10 plasma levels. All patients involved in this study were under close medical supervision prior and during the course of the study. The population study included 30 patients (14 males and 16 females) aged between 64-93, with an average age of 80.97 ± 7.47. According to the inclusion criteria, 22 patients enrolled (10 males and 12 females) aged between 64-93, with an average age of 80.59 ± 8.53. The generic CoQ10 group consisted of 12 patients (5 males and 7 females), average age 81.83±8.43 and The Ultrasome-CoQ10^{TM} group involved 10 patients (5 males and 5 females), average age 79.1±8.85.

2.2.4.2. Samples

Plasma samples for CoQ10 analysis was drawn before and at prefixed time intervals at time = 0 (prior to receiving the capsules) 1, 2, 4 and 8 hours post administration. Blood was drawn from the cubital vein, after inform consent and anticoagulated with lithium heparin. Blood samples were drawn into plastic test tube containing EDTA. Plasma was separated by centrifugation at 4000*g* for 15 min in a non-cooled centrifuge and stored at -80^{o}C until analyzed.

2.2.4.3. Description of analytic method

The method is based on oxidation of reduced CoQ10 in the sample by treating it with *para*-benzoquinone followed by extraction with 1-propanol and direct injection into the HPLC apparatus. Two hundred µL of plasma was supplemented with 50 µL of a 1,4-benzoquinone solution (2 mg/mL) and vortexed for 10 s. After 10 min 1 ml of *n*-propanol was added. The test tube was vortexed for 10 s and certifugated at 10,000 rpm for 2 min in order to spin down the protein precipitate. Two hundred µL of the supernatant was injected into the HPLC.

2.2.4.4. Analysis of CoQ10 in plasma samples

CoQ10 in plasma samples was identified and quantifies by the *Littarru* procedure (23). Briefly, The HPLC apparatus consisted of a Bekman system pump model 126, a detector model 166 (Beckman Instruments, San Ramon, CA) and an injector equipped with a 200 µL loop (Rheodyne 7725i obtained from Supleco, Milano, Italy). The column was a Supelcosil LC 18 (Supelco) 25×0.46 cm i.d. 5 µm, precolumn LC 18S, 2 cm (Supelco). An in-line filter A-701 (Upchrch Scientific, Inc. Oak Harbor, WA)

was placed between the injector and the precolumn. The photodiode array detector for the UV spectrum analysis of the CoQ10 peak was a SPD-M (Shimadzu, Tokyo). Mobile phase was constituted by ethanol-methanol (65-35%) and the flux was 1 ml/min. UV detection was performed at 275 nm.

2.2.5. Clinical trials based on Ultrasome-CoQ10^{TM} treatment

2.2.5.1. The effect of Ultrasome-CoQ10^{TM} supplementation in patients with end-stage heart failure waiting cardiac transplantation.

2.2.5.1.1. Patients and study design

Thirty two patients with end-stage heart failure awaiting heart transplantation were randomly allocated to receive either 400 mg Ultrasome-CoQ10^{TM} (60 mg of CoQ10) or placebo for 3 months. All patients continue their regular medication regimen. Assessments included anamnesis with an extended questionnaire based partially on Minnesota living with heart failure questionnaire, 6 min test walk, blood test for arterial natriuretic factor (ANF) and tumor necrosis factor (TNF) and echocardiography.

2.2.5.2. The Effect of Ultrasome-CoQ10^{TM} treatment on hip fracture rehabilitation

2.2.5.2.1. Patients and study design

The intervention group included 30 rehabilitative inpatients, hospitalized in Herzfeld hospital. The control group consisted of 43 patients with similar diagnosis, hospitalized and discharge in the preceding year. The intervention patients were monitored prospectively, using the **MDS-PAC** instrument. The same tool had previously been used for the historical control group. Daily function was assessed using standard **ADL and IADL** parameters. Additional measures tracked were stair climbing, physical endurance, pain and duration of hospital stay. All subjects in the intervention group received 400 mg of Ultrasome-CoQ10^{TM} (60 mg of coQ10) daily for the entire hospital stay. Control patients received no additional therapy. Baseline parameters and rehabilitation outcome was compared between the groups.

2.2.5.3. The effect of Ultrasome-CoQ10^{TM} treatment on chronic wounds

2.2.5.3.1. Patients and study design

A prospective demonstration study with 7 patients (Mean age of subjects was **76.9** years) who suffered from either none healing or deteriorating full thickness skin lesions for more than 20 days. Skin wounds were diagnosed as decubitus ulcers (5 wounds of this type) or skin trophic wounds (4 of this type). Patients received 400 mg/day Ultrasome-CoQ10^{TM} (60 mg of CoQ10) for 20-60 days. They were assessed

before and after treatment using **MDS** score evaluates severity of wounds including size, main tissue quality and nature of secretion. Norton scale (determines functional state of patients). In addition hemoglobin (Hb) and serum albumin (Alb) values were also assessed.

2.2.5.4. The effect of Ultrasome-CoQ10^{TM} treatment on muscles soreness and fatigue in athletes after physical activity.

2.2.5.4.1. Participants and study design

Thirty Israeli runner's athletes (21 males and 9 females, aged 25 to 56 years, average normal BMI of 21.8 kg/m^2) were involved in a randomized double blind placebo-controlled trail. The study was performed at the Hebrew university in Jerusalem, Department of nutrition and food technology, Israel. Subjects were randomly assigned to receive an oral dose of 400 mg Ultrasome-CoQ10^{TM} (60 mg of CoQ10) once daily as nutrition or placebo bar form for two periods of one week separated by a one week wash-out period. None of the participants consumed nutritional supplements, changed his diet or the regimen of his life style during the study period.

2.2.6. The effect of Ultrasome-CoQ10^{TM} treatment on neuronal damage in animal model

2.2.6.1. Study design

Male Sprague-Dawely rates (10 on each group, 250 g weight) were injected unilaterally with 12 µg of 6-OHDA into the substatia nigra (SN). The animals were tested for rotational behavior induced by injection of 0.25 mg/kg of apomorphine or 5.0 mg/kg of amphetamine starting 14 days after 6-OHDA lesion. The contralateral/ipsilateral turnings were measured visually in a round tool for a period of 120 min. The rates were orally fed daily with Ultrasome-CoQ10^{TM} as indicated.

2.2.7. Statistical analysis.

All determinations were based on at least three independent replicate samples. Results were analyzed by JMP IN statistical discovery software using one-way variance analysis (ANOVA). When a significant difference was obtained ($p<0.05$), The Tukey-Kremer HSD test was used to compare each pair of means.

3. Results

3.1. Formulation of CoQ10 in Ultrasomes

Ultrasomes are a new type of lipid particles considered as an intermediate or "Hybrid" system between liposomes and oil-in water emulsions. Ultrasome particles have a new type of lipid assembly comprising a hydrophobic core, in standard oil-in water emulsions, but surrounded and stabilized by one or more phospholipid bilayers as in liposomes. The Ultrasome technology represents a new entity as lipoidal drug vehicle and its successful development was achieved by the incorporation of a relatively high lecithin content (5-10%) compared to standard emulsions (0.5-2%), the use of fats or triglycerides which are solid at room temperature instead of oils, and the utilization of high pressure emulsification. The combination of the specific lipid composition and manufacturing technology results in the formation of stable lipid particles in the submicron range.

3.2. In vitro release of CoQ10

Figure 1 shows the *in vitro* release patterns of CoQ10 from the Ultrasome^{TM}-CoQ10 formulation and the generic CoQ10 product in simulated gastric fluid. The % release of CoQ10 from the marketed product was very low compared to a very significant release (50% after 2 hr) from the Ultrasome^{TM}-CoQ10 formulation. After capsule disruption in the stimulated gastric fluid, big aggregates or clusters of CoQ10 were observed which may explain the low CoQ10 dissolution into the release medium.

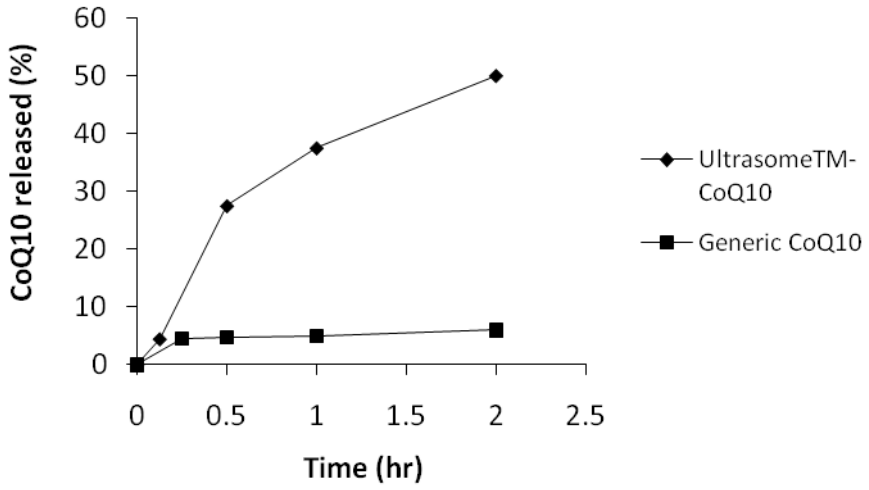

Figure 1. The *in vitro* release patterns of CoQ10 from the Ultrasome™-CoQ10 formulation and the generic CoQ10 product in simulated gastric fluid. The -CoQ10 formulation and the generic CoQ10 product contained equivalent amounts of CoQ10 (50 mg) were determined by USP apparatus type 2 (paddles) contains 8 vassels, 75 rpm filters (70 µM) In 750 ml of simulated gastric fluid (150 mM Nacl, pH=1.2, 37°C) containing 0.1% Tween 80 as sink for 2 hr.

3.3. Human oral Bioavailability results

The study group (Ultrasome-CoQ10™) showed good tolerability and no side effects were observed. After single dose administration of 90 mg of CoQ10, peaks of plasma CoQ10 levels (Cmax) appeared at 4 hours post capsule intake (tmax). At tmax the average plasma concentration among the patients who received Ultrasome-CoQ10 ™ was 1.061µg/mL whereas among the control group (those who received the generic CoQ10) it was 0.698 µg/mL **(Table 1)**. A significant higher net plasma increase was found in the group receiving Ultrasome™-CoQ10 compared to generic CoQ10. The mean absolute change (from baseline to post supplementation value) in plasma CoQ10 values was greater in Ultrasome™-CoQ10 (0.350 µg/mL) than in the control group (0.142 µg/mL) **Figure 2**. Analysis of variance (ANOVA) showed a statistically significant difference ($p<0.035$) between the two groups. This result demonstrate a 2.5 fold increase in absolute change in CoQ10 plasma concentration from baseline in patients supplemented with Ultrasome™-CoQ10 as compared with those who received generic CoQ10.

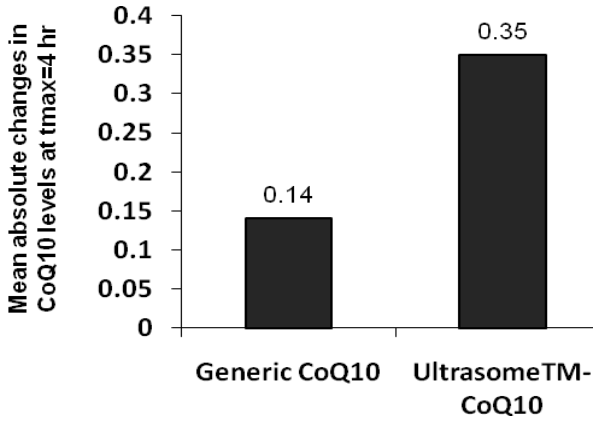

Figure 2. Bioavailability of Ultrasome™-CoQ10 compared to generic CoQ10. Differences between absolute change In Ultrasome™-CoQ10 and generic CoQ10 are statistically significant (P<0.035).

Table 1. Differences in CoQ10 plasma concentration between patients administered Ultrasome™-CoQ10 formulation and patients who received generic CoQ10.

Parameter	Generic CoQ10 (µg/mL)	UltrasomeTM-CoQ10 (µg/mL)
Mean baseline CoQ10 plasma levels (µg/mL±SE)	0.557±0.061	0.711±0.082
Mean CoQ10 levels at tmax = 4hr (µg/mL±SE)	0.698±0.085	1.061±0.112
Mean absolute change in plasma CoQ10 levels at (t4-t0) (µg/mL)	0.141*	0.350*

*Differences between absolute change In Ultrasome-CoQ10™ and generic CoQ10 are statistically significant (P<0.035).

3.4. Results of clinical trials

3.4.1. The effect of Ultrasome-CoQ10™ supplementation in patients with end-stage heart failure waiting cardiac transplantation.

Twenty-seven patients completed the study. The study group showed significant improvement in 6 min. walk test and a decrease in dyspnea, New-York heart association (NYHA) classification (cardiac damage score), nucturia, and fatigue. No significant changes were noted after 3 months of treatment cardiography parameters (dimensions and contractility cardiac chambers) or ANF and TNF blood levels.

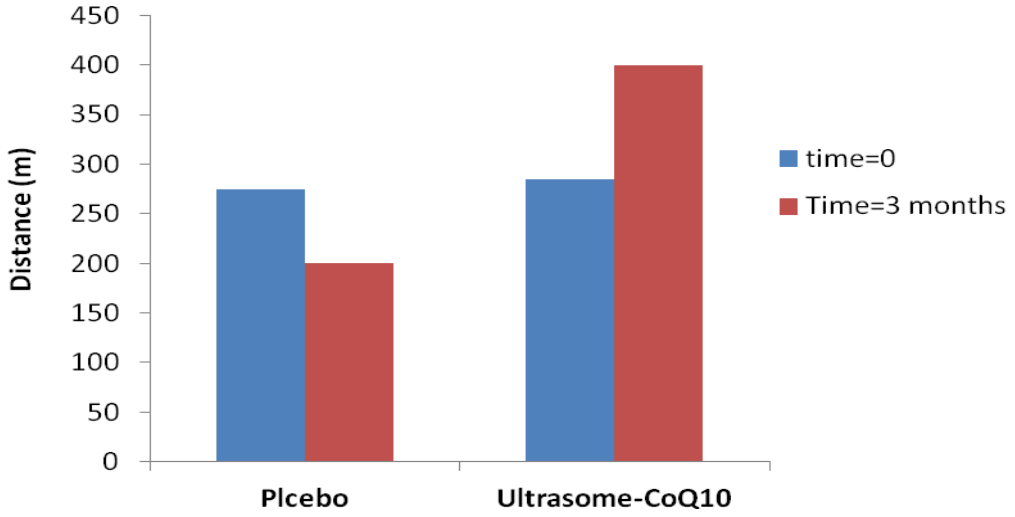

Figure 3. The effect of Ultrasome-CoQ10™ administration on six minute walk test at entry and 3 months later in the placebo and Ultrasome-CoQ10™ groups. The study group consumed 400 mg of Ultrasome-CoQ10™ (60 mg of CoQ10) daily showed a significant improvement in performance, whereas the placebo group deteriorated (p<0.0001). Median plasma levels of CoQ10 were 0.83 mg/L for the study group, and 0.178 mg/L for the placebo group.

3.4.2. The Effect of Ultrasome-CoQ10™ treatment on hip fracture rehabilitation

Base line characteristic were similar between the two groups. These include age, sex, number of diagnosis and type of surgery. Thus, selection bias was not present. Hospital stay was shorter in the intervention group 23.27 days vs. 25.49 days in control group, but the difference was not statistically significant. Mobility on discharge ($p=0.055$), the ability to dress the lower body ($p<0.01$), use of bath ($p<0.001$), the ability to prepare meals ($p<0.1$), Stair climbing ($p<0.001$), Physical endurance ($p<0.05$) was better in the intervention group compared to placebo group. In addition, pain intensity and frequency were lower in the intervention group

(p<0.001) that was supported by 400 mg of Ultrasome-CoQ10™ (60 mg of CoQ10) daily during the entire hospital-stay.

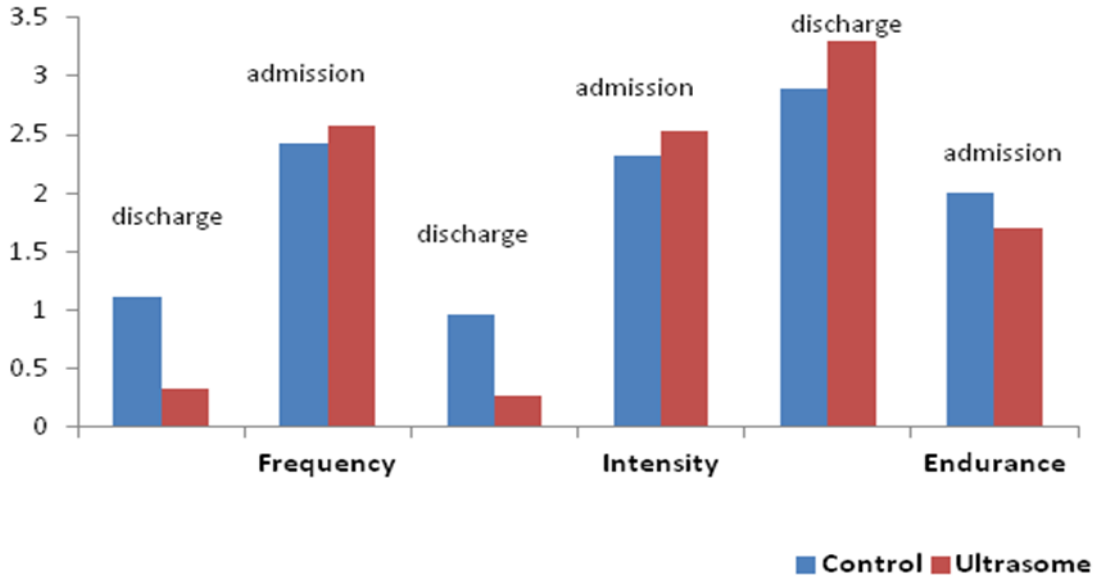

Figure 4. Influence of Ultrasome-CoQ10™ administration on frequency, intensity and endurance soreness among hip fracture hospitalized patients. Pain intensity frequency and endurance were lower in the intervention group (p<0.001) treated with 400 mg of Ultrasome-CoQ10™ (60 mg of CoQ10) daily during the entire hospital-stay.

3.4.3. The effect of Ultrasome-CoQ10™ treatment on chronic wounds

Following treatment with Ultrasome-CoQ10 measurable improvement in wounds was recorded. A lowered mean MDS score from 9.86 to 6.70 (p<0.022), wound size from 45.2 to 21.2 (p<0.068), decreased secretion from 3.2 to 1.7 (p<0.011), and main tissue quality from 1.2 to 0.7 (p<0.019). No statistically improvement was noted in hemoglobin (Hb) or albumin (Alb) values in all patients.

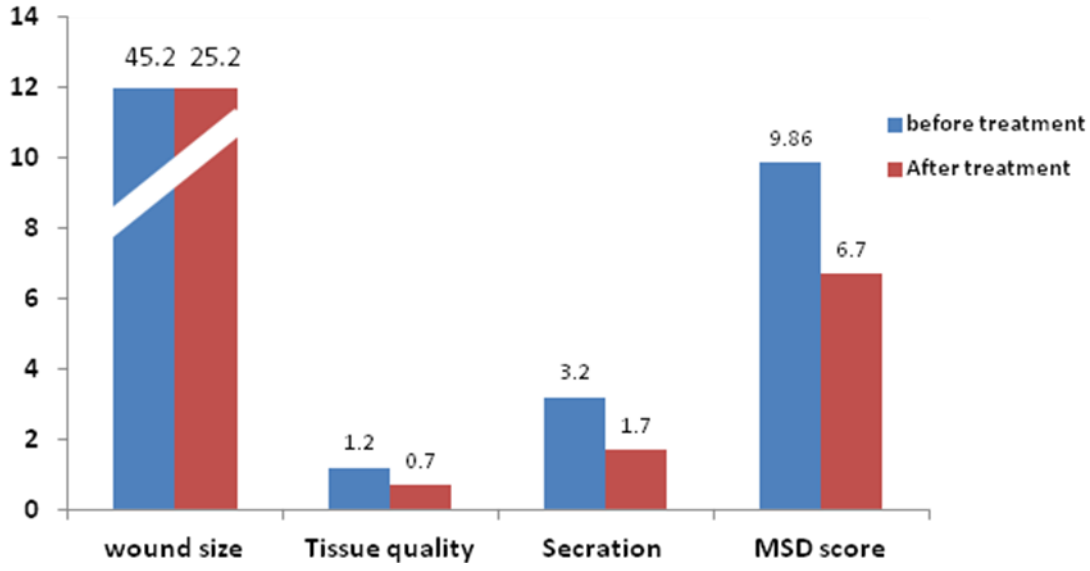

Figure 5. Influence of Ultrasome-CoQ10TM Treatment on chronic wounds. Ultrasome-CoQ10 measurable improvement in wounds was recorded. A lowered mean MDS score ($p<0.022$), wound size ($p<0.068$), decreased ($p<0.011$), and main tissue quality from 1.2 to 0.7.

3.4.5. The effect of Ultrasome-CoQ10^{TM} treatment on muscles soreness and fatigue in athletes after physical activity.

Oral administration of 400 mg of Ultrasome-CoQ10^{TM} (60 mg of CoQ10) as nutrition bar form before physical activity significantly ameliorates muscle pain and fatigue at the intervention group ($p<0.05$). 14 participants (87.5%) reported no muscle pain after physical activity. Only 2 participants suffered from muscle soreness (12.5%). In addition, at the control group the results were not statistically significant (52.9% and 47.1% respectively).

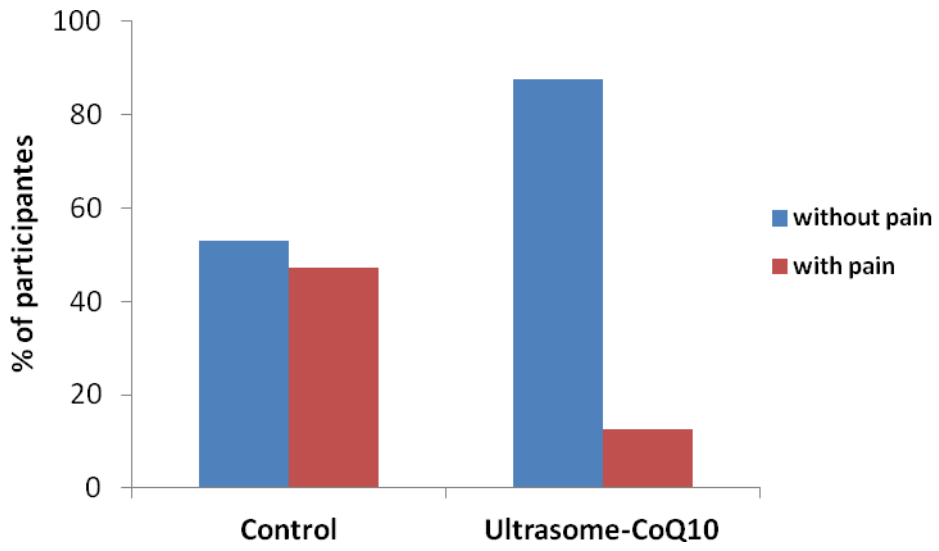

Figure 6. The influence of Ultrasome-CoQ10™ supplementation on muscle soreness among athletes. Oral administration of 400 mg of Ultrasome-CoQ10™ (60 mg of CoQ10) in nutrition bar form before physical activity for 1 week significantly reduced muscle soreness and fatigue at the intervention group ($p<0.05$). At the control group the results were not statistically significant. The study was divided to two periods of one week separated by a one week wash-out.

3.5. The effect of Ultrasome-CoQ10™ treatment on neuronal damage in animal model.

Oral administration of Ultrasome-CoQ10™ (5 mg/kg), one week before and two weeks after 6-OHDA lesion, reduced apomorphine (25 mg/kg) induced rotations by 87% (10±5, vs. 77±27 rotations/2h. $p<0.04$). The same results were obtained but with lower dosage of Ultrasome-CoQ10™ (3 mg/kg) regards to amphetamine (5 mg/kg). The reduction in rotation rate was by 72% (12±12 instead 67±18 rotations/2h $p<0.03$). When Ultrasome-CoQ10™ (3 mg/kg) was administrated for 3 days (1 day before, one day in lesion and 1 day after lesion) or for one week (first feeding 1 h lesion), the reduction in rotation rate was by 33% and 34% respectively (43±13 in the 3 days group and 42±10 in the 1 week group vs. 64±16 in the control group. $P=0.15$).

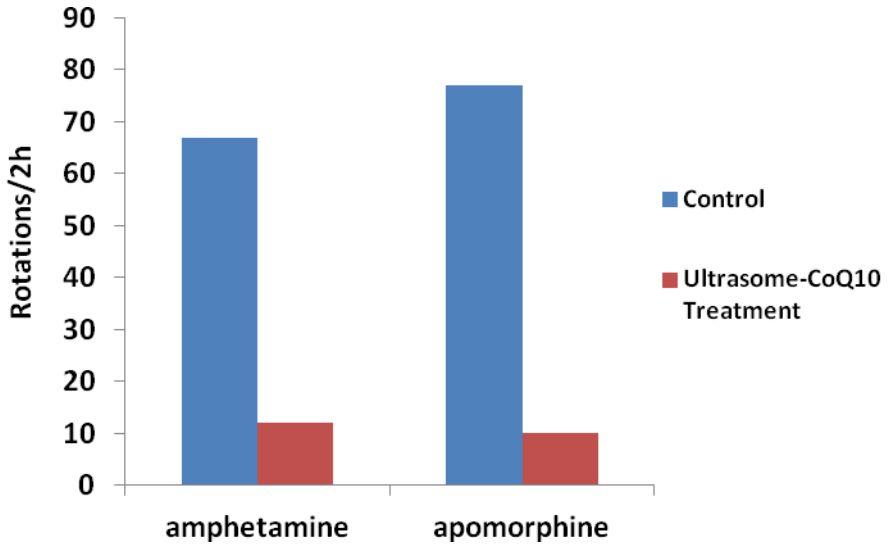

Figure 7. Influence of Ultrasome-CoQ10™ treatment on neuronal damage in animal model. Oral administration of Ultrasome-CoQ10™ (5 mg/kg), one week before and two weeks after 6-OHDA lesion, reduced apomorphine (25 mg/kg) induced rotations by 87% ($p<0.04$). The same results were obtained but with lower dosage of Ultrasome-CoQ10™ (3 mg/kg) regards to amphetamine (5 mg/kg). The reduction in rotation rate was by 72% ($p<0.03$).

4. Discussion

Coenzyme Q10 (CoQ10) is a compound found naturally in virtually every cell in the body. Adequate amounts of CoQ10 are necessary for cellular respiration and ATP production. CoQ10 also noted as an efficient intercellular antioxidant. A CoQ10 deficiency could result from impaired CoQ10 synthesis due to nutritional deficiencies such as vitamin B6, a genetic or acquired defect in CoQ10 synthesis or utilization, increased tissue needs resulting from a particular illness. On other hand CoQ10 levels may decline with advancing age and using medications such as statins. CoQ10 supplementation may benefit numerous diseases such as cardiovascular disease; muscular dystrophy; diabetes mellitus and Parkinson's disease that are linked with low levels of CoQ10. In addition, CoQ10 supplementation may benefit the quality of life of healthy subjects such as athletes and others who are doing physical exertion. CoQ10 is absorbed from the small intestine, passes into the lymphatics, and finally to the blood and tissues.

The slow and limited absorption of CoQ10 are due to its lipophilic nature, large molecular weight and low dissolution rate (24). Research of exogenous CoQ10 absorption and bioavailability varies greatly depending on formulation used. The general consensus is that slightly better absorption is achieved with oil-based forms of CoQ10 (25, 26).

Herbamed developed a unique patented Ultrsome ^{TM} technology for enhanced oral bioavailability of lypophilic compounds such as CoQ10. Ultrasomes are a new type of lipid particles considered as an intermediate or "Hybrid" system between liposomes and oil-in water (O/W) emulsions. Ultrasome particles have a new type of lipid assembly comprising a fat soluble core, in standard oil-in water emulsions, but surrounded and stabilized by one or more phospholipid bilayers as in liposomes. Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids contains polar head group covalently attached to one or two hydrophobic hydrocarbon tails. When these lipids, are exposed to an aqueous environment, interactions between themselves, (hydrophilic interactions between polar head groups and van der Walls interactions between hydrocarbon chains and hydrogen bonding with water molecules), lead to spontaneous formation of closed bilayers (27). These structures can encapsulate and effectively deliver both hydrophilic and lipophilic substances (28). Liposomes have been studied for many years as carrier system for drugs, with advantages such as enhancement of therapeutic efficacy at low dosage and, hence, reduction in toxicity of the encapsulated agent; improved pharmacokinetic profiles, enhanced tissue penetration and increased biological half life (28, 29, 30).

The Ultrasome technology represents a new entity as lipoidal drug vehicle and its successful development was achieved by the incorporation of a relatively high

lecithin content (5-10%) compared to standard emulsions (0.5-2%), the use of fats or triglycerides which are solid at room temperature instead of oils, and the utilization of high pressure emulsification. The combination of the specific lipid composition and manufacturing technology results in the formation of stable lipid particles in the submicron range.

The efficacy of the formulation was tested *in vitro* and *in vivo*. The dissolution apparatus was used for the *in vitro* assay and the oral bioavailability was tested on hospitalized, geriatric patients, undergoing continues treatment with various medications. The % release of CoQ10 from the marketed product was very low compared to a very significant release (50% after 2 hr) from the Ultrasome^{TM}-CoQ10 formulation **(Figure 1)**. After capsule disruption in the stimulated gastric fluid, big aggregates or clusters of CoQ10 were observed **(Figure 8)** which may explain the low CoQ10 dissolution into the release medium. Since particle size is a limiting factor in the rate and extent of drug absorption from gastrointestinal tract, this result indicates low oral bioavailability of CoQ10 from the commercial product compared to the Ultrasome-CoQ10^{TM} formulation (**Figure 2**).

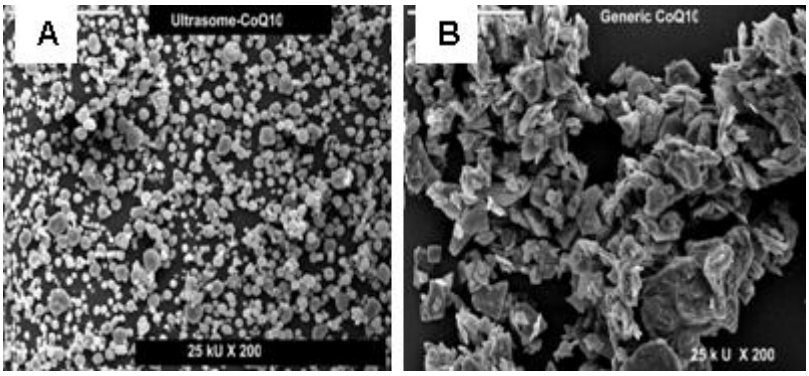

Figure 8. Particle size of Ultrasome^{TM}-CoQ10 (A) compared to generic CoQ10 (B). The particle size is one of the factors that affect the dissolution rate. Ultrasome^{TM}-CoQ10 represented by small, homogenous and uniform particles compared to large and heterogeneous particles of generic CoQ10.

The results of a human oral bioavailability study involving oral ingestion of a 90 mg of CoQ10 in encapsulated formulation (Ultrasome^{TM}-CoQ10) and the generic form are presented in **Figure 2**. The study group (Ultrasome^{TM}-CoQ10) showed good tolerability and no side effects were observed. After single dose administration of 90 mg of CoQ10, peaks of plasma CoQ10 levels (Cmax) appeared at 4 hours post capsule intake (tmax). At tmax the average plasma concentration among the patients who received Ultrasome^{TM}-CoQ10 was 1.061µg/mL whereas among the control group

(those who received the generic CoQ10) it was 0.698 µg/mL **(Table 1)**. A significant higher net plasma increase was found in the group receiving Ultrasome^{TM}-CoQ10 compared to generic CoQ10. The mean absolute change (from baseline to post supplementation value) in plasma CoQ10 values was greater in Ultrasome^{TM}-CoQ10 (0.350 µg/mL) than in the control group (0.142 µg/mL) **Figure 2.** Analysis of variance (ANOVA) showed a statistically significant difference ($p<0.035$) between the two groups. This result demonstrate a 2.5 fold increase in absolute change in CoQ10 plasma concentration from baseline in patients supplemented with Ultrasome-CoQ10^{TM} as compared with those who received generic CoQ10.

This study demonstrates that Ultrasome-CoQ10^{TM} has shown high drug-tapping efficacy and improved oral delivery of CoQ10 even in hospitalized, geriatric patients, undergoing continues treatment with various medications that may interrupt the absorption of coenzyme Q10.

The beneficial effects of physical exercise have been known for a long time. Physical exercise is associated with diverse health benefits such as reduced threat of cardiovascular disease, cancer, diabetes and in general with a lower risk of all–cause of mortality (31). However, these beneficial effects art may lose with strenuous exercise such as the ultra-marathon, cross-country running, and iron man triathlon that are becoming increasingly popular around the world (32, 33). This type of exercise causes structural damage to muscle cells indicated by muscle soreness and swelling, prolonged loss of muscle function, and leakage of muscle proteins into circulation (34). The positive effect of Ultrasome-CoQ10^{TM} was observed among Israeli athletes with respect to muscle pain and fatigue after physical activity **(Figure 6)**. Oral administration of 400 mg of Ultrasome-CoQ10^{TM} (60 mg of CoQ10) in nutrition bar form before physical activity significantly reduced muscle soreness and fatigue at the intervention group ($p<0.05$). The results were not statistically significant in the control group with respect to muscle soreness and fatigue. Diaz-Castro and co-workers reveled that CoQ10 supplementation ameliorates muscle soreness and damage (35). The potential mechanism that could be postulated is due to Ultrasome-CoQ10^{TM} higher bioavailability. The higher bioavailability influences its efficient reducing degree of oxidative stress and creatinine excretion, which would lead to the maintenance of the cell integrity. In addition, Ultrasome-CoQ10^{TM} can modulate the inflammatory signaling associate with exercise by preventing over-expression of TNF-α after the exercise, together with an increase in the sTNF-RII that limits the determinental, pro-inflammatory actions of TNF and therefore decrease muscle damage during physical performance (36).

In several clinical trials and scientific research Ultrasome-CoQ10^{TM} played a role in quality of life in patients with end-stage heart failure awaiting cardiac transplantation (5) **(Figure 3)** , in rehabilitation outcome following surgical repair of

hip fracture **(Figure 4), in** healing process of chronic skin lesions **(Figure 5)** , and in protecting against 6-hydroxydopamine induced nigra lesions in rats which indicates is potential therapy for Parkinson's disease (PD) and other neurodegenerative diseases without side effects **(Figure 7)**.

Conclusions

CoQ10 was formulated using the Ultrasome^{TM} proprietary drug delivery technology. The results of this study demonstrate the effectiveness of Ultrasome-CoQ10^{TM} with significant enhanced oral bioavailability of CoQ10 in compression to a generic CoQ10 given to geriatric patients suffering from several chronic diseases and have poor gastrointestinal absorption. Increased plasma response to supplemental treatment with Ultrasome^{TM}-CoQ10, is a clinical and statistical significance and has a positive role on quality of life.

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