

ASSESSMENTS OF INCORPORATION OF SIMVASTATIN MICROPARTICLES IN ENERGY DRINK FORMULATION FOR ENHANCING ANTI-OXIDANT AND ANTI INFLAMMATORY ACTIVITY

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ABSTRACT

The objectives of this current investigation are (1) to develop a multiple-unit microparticulate dosage form for simvastatin (50, 75 and 100 mg drug loads) from a hydrophilic polymer and a positively-charged polysaccharide moiety using a cold dispersion method, (2) to assess the anti-inflammatory and anti-oxidant properties of the energy drink formulation containing drug-loaded microparticles in an in vitro conditions,(3) Formulation of energy drink incorporating antioxidant simvastatin microparticles. Chitin and PEG 6000 were selected respectively as positively-charged polysaccharide moiety and hydrophilic polymer to generate the drug-loaded microparticles. Addition of propylene glycol led the microparticles to form a spherical-shaped matrix. A noteworthy observation came out from this investigation is that simvastatin-loaded microparticles did dissolve completely in the energy drink formulation while the pure drug did not. Even the anti-inflammatory and anti-oxidant properties of simvastatin were also retained after its incorporation into microparticles as well as its dissolution into the energy drink formulation. This result could give impetus to develop energy drink formulation possessing the re-energizing property along with the anti-inflammatory activity.

Keywords: Simvastatin, microparticles, anti-oxidant, anti-inflammatory, energy drink

No: of Figures: 1**No: of Tables: 2****No: of References: 15**

INTRODUCTION

Energy drink consumption, prior to or after strenuous physical activity is thought to be linked with instant provision of energy requirements of the body associated with exertion. These drinks have been popularized since 1987 by various commercial brands available across the globe promising greater efficiency, enhanced endurance and reduced feeling of fatigue in performers. To a certain extent these similar claims have been made and proven to be beneficial for students and long distance drivers (Malinauskas et al 2007). The origin of the energy drinks can be traced back to a Scottish drink 'Irn-Bru' in 1901. In 1929, Lucozade which was originally introduced as a hospital drink to facilitate early recovery in UK was popular for a short time. University of Florida in 1960 documented and popularized its immensely liked gators drink (derived from Gatorade) for football players. In recent years the popular energy drink brand in India, Red Bull has occupied significant share of the market sale especially amongst students ever since its launch in 1997.

Various energy drinks are being patented for their unique formulas containing hypotonic combinations salts like calcium, magnesium, zinc, sodium and potassium in addition to glucose and water. Addition of vitamins (A, B,C, E) to facilitate metabolism and increase anti-oxidant effect have been tried (Giliota, 2003). An average 250 ml of energy drink generally has about 80 mg of caffeine or other methyl xanthine derivative along with some other

ingredients in various proportions of ginseng, maltodextrin, carbonated water, inositol, carnitine etc. An Indian energy drink developed from sugarcane juice, citric acid, lemon juice, ginger extract, osmium leaf, pepper, soda and black salt has been patented by Amity University in 2009 (Amity University Indian Patent Application 2009).

Antioxidants are reducing agents which are added to pharmaceutical preparations to prevent their oxidation and retain their natural forms. Not all but only a few of the antioxidants can be added to the formulations which undergo slow oxidation and which are chemically and physiologically compatible with the product for use. Ideally such agents should be acting as preservatives also and should be readily soluble in the formulation (Chatwal, 2009). It is normally a challenge to find easy solubility of the anti-oxidant intended to incorporate in the energy drink or any pharmaceutical preparation. Converting the anti oxidant in micro particles helps in its solubility and effective fortification of the solution designed to be an energy drink.

We chose simvastatin; the methylated form of lovastatin inhibits HMG-CoA reductase. Its common use is found in its effective reduction of hyperlipidemia, LDL, triglycerides and apolipoprotein B in cases of hypertension, atherosclerosis, coronary artery disease and stroke where it is found to reduce the risk and extent of damage. Because of extensive first pass

metabolism, the bioavailability of oral simvastatin is still less than 5% and many strategies aimed at modifying release rate of the prodrug (simvastatin) or the active moiety, are under development to increase oral bioavailability (Ungaro et al 2011). It is known to cross blood brain barrier and is easily excreted through feces (mostly) or through urine.

Aims and Objectives:

The present study was conducted to devise a simple way to incorporate and to protect the antioxidant property of simvastatin, formulate an 'enriched' energy drink and evaluate the antioxidant property of the new drink. The concept of such incorporation was aimed at development of a food/drink supplement having an enhanced antioxidant property to minimize fatigue in strenuous exertion.

The objective of the present study included:

Development of a multi unit microparticulate dosage form for simvastatin (50,75, 100 mg drug loads) from a hydrophilic polymer and a positively charged polysaccharide moiety using a cold dispersion method.

Evaluation of anti-inflammatory and anti-oxidant properties of the drug loaded microparticles in an in vitro condition. Formulation of energy drink incorporating antioxidant simvastatin microparticles.

Material and Methods:

Simvastatin in its pure form was procured from Kwality Pharmaceuticals Amritsar,

Punjab. The purity of the drug was confirmed by conducting different preformulation studies which include melting point determination, FT-IR, TLC and UV spectrophotometry. Aqueous solubility enhancement studies (Affandi et al, 2016) were conducted at different temperature conditions using PEG6000 either alone or in combination with chitin. Phase solubility studies were carried out by mixing various concentrations of solubility enhancer molecules (chitin and PEG6000) in the saturated drug solution prepared in 20ml of distilled water. The mixtures were shaken on a water bath shaker for 5 hours at 60rpm. The mixtures were then filtered and the drug concentrations were determined spectrophotometrically to calculate the solubility efficiency as per method described by Affandi et al (Affandi et al 2016)

Preparation of Micro particles:

Simvastatin loaded micro particles were prepared using a cold dispersion modified technique as reported by Jaspert et al, 2005. The oil phase was developed by mixing 150 ml of coconut oil, 0.1 ml of propylene glycol along with 2 drops of Span 80 which are kept cool in for 30 minutes in a refrigerator. The lipid phase was prepared by melting PEG 6000 (polyethylene glycol) and chitin together at 60-65 deg C using water bath. The lipid phase was transferred into oil phase while stirring at a speed of 750-1000 rpm in an electrical stirrer. Continued stirring for 1 hr 30 min yielded spherical micro particles. Once formed, Microparticle solution was mixed with 150 ml of n-Hexane drop by

drop while the stirring continued to rigidize the structure. The rigid micro particles were sieved using Endecott's mechanical shaker (Endecott's Ltd, London UK) through #30 sieve (500 microns), dried overnight under ceiling fan at room temperature and were finally packed in a plastic pack and stored

in a desiccator. Similar procedure was followed for preparation of simvastatin loaded microparticles except in the lipid phase preparation where three types of solutions were prepared by adding simvastatin in varying amounts (50,75 and 100 mg) while melting PEG 6000 and chitin.

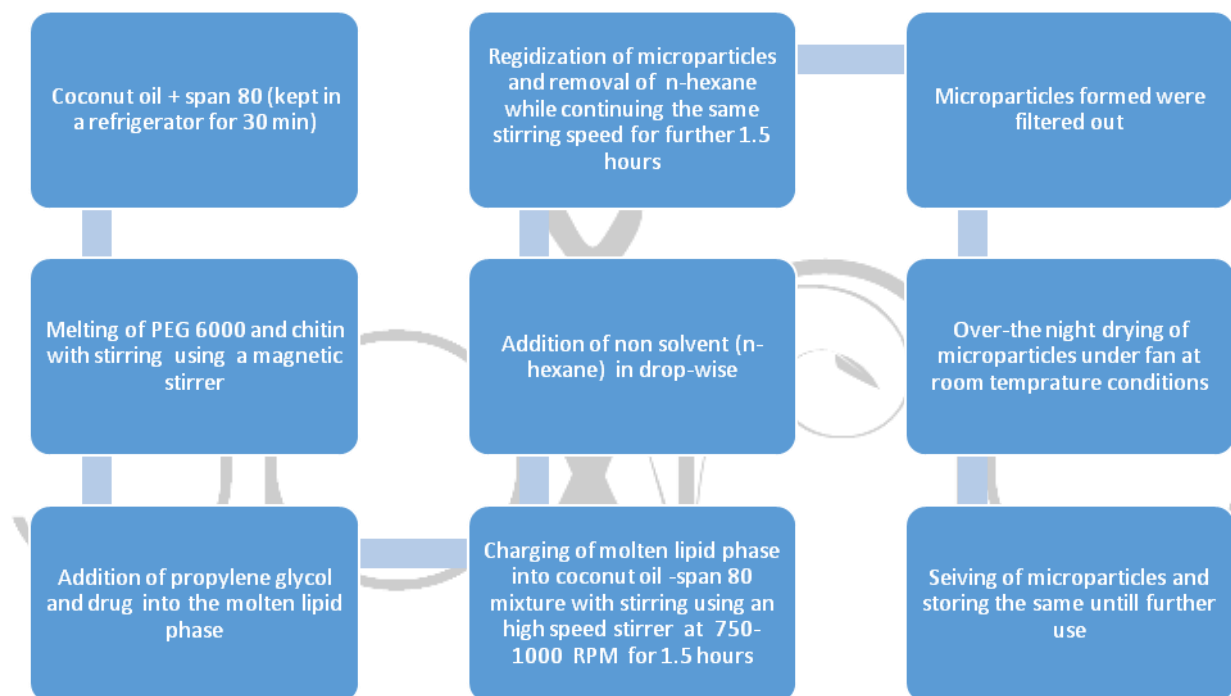


Fig. 1: Method followed for preparation of PEG-Loaded Simvastatin Microparticles

In total, 1+3 formula were tried to obtain microparticles having higher yield percentage and good drug entrapment efficiency. Inclusion of chitin and propylene glycol resulted in the formation of spherical PEG based microparticles. This

layer chromatography study was done to find out retention factor value for pure simvastatin and for simvastatin loaded microparticles. These microparticles were then subjected to Fourier transform infrared (FT-IR) spectroscopy to determine and

compare the observed wave number values for various functional groups present in simvastatin pure drug powder and in drug loaded microparticles. This test was done to find out drug structural modification within the microparticles.

The microparticles thus prepared were tested for antioxidant activity and for anti-inflammatory effect.

Preparation of Energy drink formulation:

Three sets of blank (without drug loading) and loaded (simvastatin Microparticle loaded) each was prepared as per the description of the ingredients given below. The contents are same as commercially available energy drinks which have proven effects and are approved for human consumption.

Table 1 Ingredients used to prepare energy drink formulation blank

Ingredients	F ₅	F ₆	F ₇
Sucrose (g)	33.75	0.0	38.75
Glucose (g)	0.0	33.75	0.0
Sodium citrate (g)	0.225	0.050	0.050
Sodium benzoate (g)	0.0	0.0	0.200
Benzoic acid (g)	0.0	0.2	0.0
Flavouring agent (g)	0.1	0.1	0.1
Microspheres (g)	0.0	0.0	0.0
Distilled water (ml)	250	250	250

Table 2 Ingredients used to prepare simvastatin loaded energy drink formulation

Ingredients	F ₈	F ₉	F ₁₀
Sucrose (g)	33.75	0.0	38.75
Glucose (g)	0.0	33.75	0.0
Sodium citrate (g)	0.225	0.050	0.050
Sodium benzoate (g)	0.0	0.0	0.200
Benzoic acid (g)	0.0	0.2	0.0
Flavouring agent (g)	0.1	0.1	0.1
Microspheres (g)	0.1	0.1	0.1
Distilled water (ml)	250	250	250

Procedure to make energy drink:

The required amount of sucrose was dissolved in 250 ml of distilled water and shaken on a magnetic stirrer at 700 rpm till completely dissolved. To this solution the benzoic acid, sodium citrate, vanillin and coloring agents were added in the required amounts mentioned in the above tables and the contents were mixed till dissolved. After all the excipients were dissolved in the solution, 100 mg of microspheres were dissolved in the energy drink formulation. The solution was then filtered and stored in an airtight container.

In vitro antioxidant activity- DPPH (2,2 diphenyl-1-picrylhydrazyl) scavenging activity

After the incorporation of simvastatin loaded microspheres, the antioxidant

activity of the energy drink formulation was tested. For this test 10 mg of drug loaded microspheres (equivalent to 8 mg simvastatin) were dissolved in 10 ml of the formulation. Half of this solution (Test) was mixed with 1 ml of methanol and 6 ml of DPPH methanolic solution (Kandappa et al, 2015). The absorbance value of this solution was measured by calculation of percentage of free radical inhibition by control sample and test solution by the following formula

$$I \% = \frac{(A_c - A_s)}{A_c} \times 100 \dots \dots \text{eq 1}$$

Where A_c is the absorbance of the control reaction (containing all reagents except test compound) and A_s is the absorbance of the reaction mixture containing the test compound (Mohamadin et al, 2011).

Preparation of methanolic solution of DPPH was done using 5mg of DPPH powder dissolved in 100 ml of methanol to produce 0.006% w/v DPPH methanolic solution (Kandappa et al ,2015). Standard sample solution was prepared using 5 mg of L-ascorbic acid dissolved in 1 ml of methanol and 6 ml of DPPH methanolic solution. The control solution used was prepared by dissolving 5 mg of pure simvastatin in 1 ml of methanol and 6 ml DPPH methanolic solution.

In vitro anti-inflammatory assessment (Protein denaturation bioassay test):

In this assay, 10 mg of drug loaded microparticles, (equivalent to 8 mg of simvastatin) was dissolved in 10 ml of energy drink formulation. Different volumes of energy drink solutions were mixed with 0.2 mg of egg albumin and 2.8 ml of buffer solution so that the final drug concentrations varied from 8-40 µg/ml. Diclofenac sodium solution was selected as reference standard and the reference solutions for comparison were prepared as per similar process as quoted above. All the reaction mixtures were incubated at 37±2 deg C in an incubator for 15 min followed by heating them to 70 deg C for 5 min in a water bath. The absorbance for all the reaction mixtures was measured at 660 nm in a spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = \left[\frac{v_t - v_c}{v_c} \right] \times 100 \dots \dots \text{Eq 2}$$

Where, V_t = absorbance of test sample; V_c = absorbance of control.

Results:

The intrinsic solubility of simvastatin in distilled water was found to be 0.071+ 0.001 mg/ml. No progressive enhancement in the solubility of simvastatin was noticed with increase in the concentrations of PEG6000 with or without chitin. This observation is in contrast with findings of earlier study by Affandi et al, 2016. The yield percentage value for different microparticles of simvastatin was found to be in the range of 76% indicating that the selected method to prepare microparticles was satisfactory. Microparticles prepared by using formula 3 (F3) was observed to have highest drug entrapment efficiency (DEE) value (98.88 +0.012) which was more than those for F2 (75.26 +0.01) and F4 (80.15 +0.02).

The retention factor observed in this study on thin layer chromatography study (TLC) for pure simvastatin was similar to values observed for simvastatin loaded microparticles. This result showed that neither the drug degradation nor the drug excipient interaction have occurred within the developed microparticles. FT-IR performed on the prepared microparticles and pure drug yielded similar wave number values indicating that no drug structural modification had occurred within the microparticles.

Limitations of laboratory animals use constrained us to perform the anti oxidant and anti-inflammatory effectivity test in vitro which are objective methods of documentation. Prevention of protein denaturation by medicinal agents in an in vitro condition is an indication of anti inflammatory effect of simvastatin. In our study microparticles with 75 mg drug load

were used for in-vitro anti-inflammatory assay. At 16 µg/ml there was a positive value in the percentage inhibition of protein denaturation for tested microparticles while the reference solution showed the % inhibition of protein denaturation with negative sign. This indicates the superiority of drug loaded microparticles to produce anti-inflammatory activity at relatively lower concentration range in comparison to the reference standard used.

To show the anti-oxidant activity of the selected drug simvastatin, the DPPH scavenging activity test was chosen as per study done by Mohamadin et al (2011). Since DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable molecule, the reduction capacity of DPPH radicals was determined by decrease in the its absorbance at 517 nm. The % inhibition in all the test solutions was less than the standard solution (ascorbic acid). The microparticles prepared thus pass the DPPH scavenging activity test (Kandappa et al, 2015)

The antioxidant and anti-inflammatory properties of the energy drink formulation in blank and simvastatin micro particles loaded samples were tested. It was observed that the percentage of free radical inhibition as calculated by (eq 1) and percentage of inhibition of protein denaturation using (eq 2) were less in test samples as compared to reference samples which indicate that the energy drink formulation possesses significant anti oxidant and anti- inflammatory activity.

Discussion:

Natural antioxidant molecules like retinoids, tocopherols, ascorbic acid and synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate are routinely added into energy drink formulations. The statin group of drugs like simvastatin also has similar properties against lipid peroxidation in addition to its anti inflammatory and hypolipdemic effect (Matalaka et el 2013). They are well documented to be associated with reduction of cardiovascular morbidity and mortality (Eisenberg et al, 1998). However, effective incorporation of the antioxidant drug enhancing its solubility while retaining the structure or non-reaction with the excipients remains a challenge. Equally important would be to maintain a dose per serving (usually 250 ml) safe enough to avoid all the adverse effects and well within the domain of FDA approval. The spade work and chemical reactions involved have been well researched and documented (Affandi et all, 2016, Kang et al 2004) and the present study used the same methodology to achieve its aims which included complex process of microparticle formation and testing for antioxidant and anti-inflammatory effects. There were no major deviations from the studies conducted by Affandi et al except that in the present study we did not find progressive enhancement in solubility of simvastatin with increasing concentrations of PEG 6000 or chitin which was found by Affandi et al and was attributed to different extent of arginine interaction with simvastatin mainly by altering the drug solvation.

FT-IR spectroscopy, a method of characterization of drug and drug loaded

particles, was shown to have similar wave number values in another study conducted by Kupcewicz et al, 2013. The validated analytical method for quantitative determination of simvastatin has the advantage of cost effectiveness and absence of polluting reagents. All the validation parameters in the present study were found to be highly satisfactory which included accuracy, precision, linearity, LOD and LOQ. The beneficial effects of simvastatin make it a suitable agent to be incorporated in energy drinks in the form of drug loaded microparticles. There would be two options to do so. Either the microparticles can be added at the level of the manufacturer in a soluble form having a fixed dose or the microparticles can also be provided with blank energy drink in a sachet for the consumer to dissolve it prior to consumption.

Conclusion:

Fortification of a food drink/energy drink formulation with simvastatin loaded microparticles impart significant anti oxidant activity and anti-inflammatory activity to the beverage as tested by in vitro DPHH activity and protein denaturation bioassay tests. Possibility of exploiting this property of simvastatin in marketing of preventive health drinks especially in obese, coronary artery disease and atherosclerotic or sedentary individuals holds a promising future. The antioxidant and anti-inflammatory activity will boost the performance and delay fatigue in people engaged in moderate to strenuous exertion.

Conflicts:

There are no conflicts with the present study done at the School of pharmaceutical sciences, Lovely Faculty of Applied Medical Sciences, LPU, Punjab.

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