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Evaluation of garlic oil in nano-emulsified form: Optimization and its efficacy in high-fat diet induced dyslipidemia in Wistar rats

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ABSTRACT

Garlic oil nanoemulsion was formulated using ultrasonic emulsification and the optimized garlic oil nanoemulsion ratio (1:2) of oil: surfactant showed spherical, with tiny droplet size 24.9 ± 1.11 nm. It was observed that the prepared nanoemulsion has the zeta potential of −42.63 ± 1.58 mV and a low poly-dispersity index of 0.2 ± 0.09 with excellent stability. The formulation was subjected to in vivo acute and sub-acute toxicity. In acute toxicity study, single oral administration of 18.63 ml of garlic oil nano-emulsion/kg resulted in immediate mortality. However, garlic oil nanoemulsion (0.46 ml/kg) and tween 80 (0.5 ml/kg) administered rats did not exhibit any toxicity and showed no changes in hematological and histological parameters. Further, both preventive and curative studies of garlic oil nanoemulsion were evaluated in high-fat diet fed dyslipidemic Wistar rats. Garlic oil nanoemulsion administered groups showed a significant effect in reducing the levels of lipid profiles ($p < 0.001$) compared to atorvastatin and garlic oil. Evaluation of lipid deposits in hepatic tissues was analyzed by Oil Red O staining, which revealed that garlic oil nanoemulsion administered rats markedly reduced the fat depots. Our findings suggest that garlic oil nano-emulsified form reduced toxicity and improved efficacy in preventing and treating dyslipidemia.

1. Introduction

Dyslipidemia, in the present scenario, is one of the most common risk factors worldwide, which is associated with the development of complications like coronary heart diseases, atherosclerosis, stroke, and ischemic heart diseases, which are also the major reasons for mortality (Shin et al., 2014; Chu et al., 2015). Dyslipidemia is characterized by excessive fat accumulation that is stimulated by unhealthy diet consumption and sedentary lifestyle causing raised levels of total cholesterol, LDL, VLDL, and reduced HDL in the blood (Durrington, 2003; Lu et al., 2014; Han et al., 2015).

Medications including statins such as atorvastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, and simvastatin; fibrates such as gemfibrozil and fenofibrate; niacin were developed in controlling lipid profiles (Kobayashi et al., 2008; Wat et al., 2016). However, all statins are associated with many adverse effects, including myopathy, myalgia, rhabdomyolysis and increased levels of hepatic enzymes (Giorgi et al., 2011; Shin et al., 2014; Chu et al., 2015). Recently, the cholesterol-reducing drug, tredaptive was introverted from the market due to its high toxicity and low efficacy (Krentz, 2013). Several researches are investigating few natural drugs for hyperlipidemia that could inhibit cholesterol production in the liver, reducing serum low-density lipoprotein cholesterol levels (Han et al., 2015).

Garlic (*Allium sativum* L.; Alliaceae) is a popular spice in cooking and widely used as a medicinal herb across the globe. Garlic and its components have a variety of beneficial biological activities (Karuppiah and Rajaram, 2012). Several organosulfur compounds that are present in garlic oil have been shown to possess numerous biological activities, in which the three most important organo-sulfur compounds include diallyl sulfide, diallyl disulfide, and diallyl trisulfides (Gao et al., 2013). The beneficiary activities of garlic oil include cardiovascular protective, antilipidemic, hypotensive, antithrombotic, antiplatelet aggregation, immunomodulatory and antitumor effects (Amagase, 2006; Corzo-Martínez et al., 2007;

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Huang et al., 2013; Li et al., 2014). However, use of garlic oil in medicine, food, pharmaceutical industry and cosmetics is very limited due to its lipophilic characteristics, volatility, strong odour and low stability in the gastrointestinal fluids, which lowers its bioavailability for systemic circulation after oral administration (Gao et al., 2013; Phadatare et al., 2014).

Currently, several researches focus on the development of lipid-based formulations, to enhance the solubility and oral bioavailability of poorly water soluble drugs, on passage through the gastrointestinal tract (Subramanian and Ghosal, 2004). One of the most promising technologies for drug delivery is by incorporating the active lipophilic component into inert lipid vehicles such as oils, dispersions of surfactant, self-emulsifying formulations, microemulsion, nanoemulsion, self-microemulsifying formulations, and liposomes. Such formulations are used to increase solubilization behaviour of drug, thus increasing the interfacial area of medications enhancing its absorption through the mucosa (Gorain et al., 2014; Gumus et al., 2015; Ferreira et al., 2016).

Nanoemulsion is colloidal dispersions of two immiscible liquids such as oil, surfactant, and cosurfactant at different ratios. Nanoemulsion are isotropic, transparent or translucent systems having a droplet size of 20–600 nm depending on the amount of surfactant and mechanical energy (McClements, 2012a; Fornaguera et al., 2015). Nanoemulsion is thermodynamically and kinetically stable, which provides ambient thermodynamic stability against aggregation, flocculation, coalescence, and Ostwald ripening due to nanometer droplet size range (Mason et al., 2006; McClements, 2012b; Jaiswal et al., 2015).

In vivo studies on the toxic effects of higher doses and prolonged consumption of garlic oil have been discussed, however, there are no reports of the nano-encapsulated garlic oil toxicity and efficacy (Kasinath et al., 1997; Zhao et al., 2013). Therefore, the aim of this study was to evaluate the physicochemical properties of formulated garlic oil nanoemulsion and the toxicity profiling was examined by *in vivo* acute and sub-acute toxicity studies. Further, the therapeutic efficacy of garlic oil nanoemulsion in preventing and treating dyslipidemia was investigated in HFD supplemented experimental Wistar rat model.

2. Materials and methods

2.1. Chemicals

Garlic oil blend (Diallyl disulfide 30–50%, Diallyl trisulfide 10–13%, Allyl sulfide 5–13%) purchased from Sigma-Aldrich, (Bengaluru, India). Tween80 was purchased from Sisco Research Laboratories, Pvt. Ltd., (Mumbai, India). The diagnostic kits used for the estimation of serum parameters were purchased from Span Diagnostics Ltd., (Gujarat, India).

2.2. Nanoemulsion formulation

Nanoemulsion was formulated using two-step procedure, initially coarse emulsion was prepared by mixing the oil and surfactant (Tween80) in different ratios (1:1:8, 2:1:7, 3:1:6, 1:2:7) followed by addition of Millipore water (Pall Corporation, MA, USA) using magnetic stirrer (REMI Instruments, Vasai, India) at 600 rpm for 10 min at 25 °C. Then, prepared coarse emulsion was further emulsified using a 20 khz Sonicator (Ultrasonics, Newtown, USA) with a maximum power output of 750 W under chilled water, (3.5 °C) which was passed continuously through this jacket to avoid a rise in temperature. The sonication process was carried out at 10 min and 20 min time intervals and formulated nanoemulsion was characterized. After the stability analysis, the nanoemulsion was used for further studies.

2.3. Characterization of nanoemulsion

2.3.1. Particle size distribution

The mean droplet size and polydispersity of the nanoemulsion were determined using SZ-100 Dynamic Light Scattering (DLS) (Horiba Scientific, Kyoto, Japan). Samples were diluted with deionized water to avoid the effects of multiple scattering. The results reported as the average diameter (*z*-average mean) and width (Polydispersity Index- PDI) was calculated from the diffusion coefficient using the Stokes – Einstein equation

$$D_h = \kappa T / (3\pi\eta D_m)$$

D_h –Particle Hydrodynamic size, κ – Boltzmann constant, T - Thermodynamic temperature, η- Viscosity, D_m - Particle diffusion coefficient. All the measurements were made in triplicate at 25 °C.

2.3.2. Zeta potential analysis

The surface charge of the nanoemulsion was measured using (SZ-100, Horiba Scientific, Kyoto, Japan) by Laser Doppler electrophoresis. The samples were diluted with deionized water and injected into a capillary cell for charge measurement at 25 °C, and the measured zeta potential values expressed in mV.

2.3.3. pH measurements

The pH of freshly prepared nanoemulsions was measured using (HI 2215, Hanna Instruments Inc., Woonsocket, USA) pH meter.

2.3.4. Transmission electron microscopy (TEM)

The morphology and structure of garlic oil nanoemulsion were examined using transmission electron microscopy (FEI-TECNAI G2-20 TWIN, Netherland). The formulations were diluted at 10 and 100 fold with deionized water and few drops of the diluted samples were placed in a carbon film-coated 300 mesh copper grids. The grid was allowed to dry under vacuum condition for 3 h and examined by transmission electron microscopy at 80 kV.

2.3.5. Stability analysis

Initial stability of prepared nanoemulsion was evaluated by centrifugation (REMI Instruments Vasai, India) at 5000 rpm for 30 min. The stable formulation did not show any turbidity or phase separation and sedimentation. The long-term stability was evaluated based on optimized nanoemulsion stored at 25 ± 2 °C, and 40 ± 2 °C. The stored samples were subjected to droplet size, PDI and pH measurement. All measurements were done in triplicates, and the results were reported.

2.4. In vivo toxicity assessment of garlic oil nanoemulsion

2.4.1. Animals and experimental design

Healthy adult female and male albino Wistar rats (8–12 weeks, weighing 100–120 g) were used for the study. All animal maintenance and experiments were carried out according to the ethical guidelines suggested by the Institutional Animal Ethics Committee of the VIT University, Vellore (Registration No – VIT/IAEC/11th/Oct 10th/No.11). Animals were housed in polypropylene cages and maintained under standard conditions of 12 h dark/light cycle at 27 ± 1 °C. The rats were supplied with regular pellets and water *ad libitum*.

2.4.2. Acute oral toxicity study

Acute oral toxicity study of GNE for calculating LD50 range was performed based on Organization for Economic Co-operation and Development (OECD) guidelines 423 (2001). Based on OECD recommendation, calculation of LD50 range was carried out in