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Oral microemulsion of phytoconstituent found in licorice as chemopreventive against benzo(*a*)pyrene induced forestomach tumors in experimental mice model

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ABSTRACT

Dibenzoylmethane (DBM), a minor phytoconstituent found in roots of licorice (*Glycyrhiza glabra*) has been reported to exhibit antioxidant and chemopreventive effects. It suffers from a problem of poor aqueous solubility and permeability leading to low oral bioavailability. Microemulsion, a novel colloidal carrier was proposed to improve its solubility in order to achieve enhanced oral biodistribution and efficacy. The microemulsion was prepared using peppermint oil and Tween 20 as oil phase and surfactant respectively. The spherical globules of microemulsion depicted a mean globule size of 157 nm and polydispersity index (PDI) of 0.715. The results of *exvivo* intestinal permeation using *non everted* intestinal sac technique demonstrated 5.7 time enhancement in intestinal permeability from microemulsion. During the *invivo* chemopreventive evaluations using benzo(*a*)pyrene [B(*a*)P] induced forestomach tumors in mice model, the treatment with DBM microemulsion, resulted in almost 100% reduction in tumor incidence after the last dose of B(*a*)P. The histopathological studies suggested the regression of stomach tumors after treatment with DBM microemulsion. Also, the biochemical estimations for oxidative stress markers depicted its improved efficacy in chemoprevention. The above results suggested that oral microemulsion formulation augmented the permeability and effectiveness of DBM as potential chemopreventive agent.

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1. Introduction

The biological source of licorice is the roots of the plant *Glycyrrhiza glabra* belonging to family Leguminosae. Licorice roots are used as natural and herbal supplement for medicinal purposes. Various chemical constituents in licorice are known to reduce inflammation, thin mucous secretions, decrease cough and increase body's ability to heal ulcers. Among various components of licorice, the major one i.e., glycyrrhizin is well known and used widely in folklore medicine for its potential benefits. The main focus of this manuscript is on one of the minor constituent found in the root extract, i.e., dibenzoylmethane (DBM) which is known to exhibit promising activities viz., chemopreventive, antioxidant, antimutagenic, UV-A radiation absorber etc [1]. As per literature reports, feeding 1% DBM in mice diet, inhibited the occurrence of mammary tumor induced by DMBA, which is immunosuppressor and a potent carcinogen (7,12-dimethylbenz(*a*)anthracene). The tumor multiplication was also reduced by 97%. Moreover, the incidence of other cancers like lymphomas/leukemias was fully attenuated by using 1% DBM in diet [2]. Further, DBM has been reported to exhibit anti-tumorigenic, and chemopreventive activities in various animal models against mammary [3], prostate [4], colon [5], skin [6] and lung cancer [7]. Cytotoxic potential of DBM in squamous cell carcinoma (HSC-2) and leukaemia cells have also been reported [8]. It is also a very potent antimutagenic agent. Its antimutagenic potential was studied using Ames *Salmonella*/microsome assay, a short term assay for studying genotoxicity potential [9]. DBM is included in Generally recognized as safe (GRAS) list by Flavor & Extract Manufacturers Association (FEMA)

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in 1965 and its food use is approved by FDA under the reference 21 CFR 121. 1164. The Council of Europe included DBM at 2 ppm, in the list of admissible artificial food flavorings during the year 1970.

Structurally, DBM is a β-diketone and a structural analogue of curcumin, the yellow pigment of turmeric. Both DBM and curcumin contains a "central β-diketone group" in association with carbon chain (unsaturated) which tends to increase enolization of the β-diketone group. But DBM has more potent antimutagenic action as compared to curcumin molecule owing to its small size and absence of hydroxyl groups on the aromatic rings [9]. However, DBM is also practically insoluble in water. *In vivo* pharmacokinetic study in rats have shown only 11.5% bioavailability of DBM indicating low absorption in the intestine or extensive metabolism in gut/liver [10]. In order to overcome the problem of poor solubility and low bioavailability, a microemulsion system of DBM was proposed.

The term 'microemulsion' is referred to as thermodynamically stable, clear and isotropic colloidal dispersions consisting of oil and aqueous phase stabilized by surfactant molecules. Sometimes a cosurfactant is also used along with surfactant. They have emerged as potential delivery systems for substances with poor solubility, toxic potential and inability of the substance to reach the site of action [11]. Microemulsions are potential drug carriers capable of carrying hydrophobic as well as hydrophilic drugs. These unique systems have attracted the attention of formulators over the past few decades, due to their properties like high solubilisation capacity, low interfacial tension and greater interfacial area. These adaptable delivery systems protect the drug molecule against oxidation, enzymatic hydrolysis. Moreover, they remarkably improve the solubilization of lipophilic drugs to improve their bioavailability. Microemulsions have been extensively studied for protection of biodegradable drugs, e.g., proteins and peptides through biological environment of peroral route [12].

The present study was aimed at the development of microemulsion based delivery system of DBM to enhance its oral bioavailability for the treatment of forestomach pappilomas. *Ex vivo* permeation studies were performed using *noneverted* rat intestinal sac model for 24 h. *In vivo* pharmacodynamic assessment of chemopreventive potential was performed against B(*a*)P-induced forestomach tumors in the experimental mice model.

2. Materials and methods

2.1. Materials

DBM was purchased from Sigma Aldrich, USA. Peppermint oil was purchased from Ajanta Chemicals Co., Gurgaon, India. Tween-20 was purchased from S.D. Fine Chemicals, Mumbai, India. Benzo(*a*)pyrene, Sodium dodecyl sulphate and Phosphotungstic acid was purchased from Sigma Aldrich, Bengaluru, India. Thiobarbituric acid, 5-5′- dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) were obtained from Hi Media Laboratories Pvt., Mumbai, India. Pyridine, Copper sulphate and Sodium hydroxide (Biuret regaent) and n-butanol were obtained from Fischer Scientific, Mumbai India. All other reagents and chemicals used were of analytical reagent grade.

2.2. Animals

Approval to carry out *ex vivo* and *in vivo* studies in animals was obtained from the Institutional Animal Ethics Committee, Panjab University, Chandigarh and their guidelines were followed throughout the studies. The Albino female mice (Balb/C strain) of 3–4 weeks old, weighing 20–30 g were used for *in vivo* evaluation and male Wistar rats weighing 250–300 g were used for *exvivo* permeation studies. The animals were kept in CAH (Central Animal House) and were kept under standard 12/12 light/dark cycle with food and water *ad libitum*.

2.3. Construction of ternary phase diagram

The ternary phase diagram was constructed by titration of uniform liquid mixtures of oil, surfactant, with aqueous phase at room temperature. Peppermint oil, Tween 20 and triple distilled water were used as oil phase, surfactant and aqueous phase respectively.

The uniform blends of oil-surfactant were prepared, where contents of oil and surfactant in the mixtures were varied from 9:1 to 1:9. The aqueous phase was added dropwise to each oily mixture kept on magnetic stirrer to allow equilibration between the phases. The mixture was visually examined for transparency after addition of each drop of water. Transparent, single-phase mixtures were designated as oil-in-water microemulsions [13].

2.4. Preparation of DBM formulations

DBM loaded o/w microemulsion was prepared by dissolving DBM in peppermint oil followed by addition of Tween 20 with the aid of magnetic stirrer. Finally, water was added drop wise to obtain a clear microemulsion spontaneously with continuous magnetic stirring (Table 1).

DBM was also incorporated into emulsion and aqueous dispersion to compare its permeation across intestinal mucosa from different formulations.

An emulsion was prepared by dissolving the DBM (1% w/w) in peppermint oil followed by addition of 20% w/w Tween 20. This mixture was triturated in a mortar and pestle with the addition of triple distilled water to get o/w emulsion.

An aqueous dispersion of DBM was prepared using 0.5% w/w sodium carboxymethylcellulose. The conventional emulsion and aqueous dispersion of DBM were used as controls in *ex vivo* intestinal permeation studies.

2.5. Globule size and zeta potential

Physical characteristics like globule size distribution and PDI were estimated by using the dynamic light scattering (DLS) phenomenon. Analysis of zeta potential and PDI of the developed formulation was done using Delsa Nano Particle Analyser (Beckman Coulter, USA). The zeta potential estimation was done at ambient temperature and electric field strength of 23.2 V/cm using zeta cell [14].

2.6. Morphology and structure

Morphology and structure of microemulsion was determined with the aid of Transmission Electron Microscopy (TEM) at CIL, Panjab University, Chandigarh. One drop of diluted samples was placed on a carbon-coated copper grid followed by addition of

Table 1
The composition of developed DBM emulsion and DBM microemulsion.

Ingredients	Composition (g)	
	Emulsion	Microemulsion
DBM	0.1	0.1
Peppermint oil	2	1
Tween 20	2	4
water	5.9	4.9

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