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Assessment of pharmacokinetic parameters of lupeol in *Ficus religiosa* L. extract after oral administration of suspension and solid lipid nanoparticles to Wistar rats

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ABSTRACT

In spite of wide applications of herbal drugs as phytochemicals or extracts, their application is limited because of their poor bioavailability. The aim of the present work was to enhance the poor bioavailability of lupeol present in *Ficus religiosa* L. extract through solid lipid nanoparticles (SLN). *Ficus religiosa* L. extract loaded SLN were prepared and administered orally to male Wistar rats for bioavailability studies. Developed SLN were characterized for particle size, PDI, zeta potential, entrapment efficiency, *in vitro* drug release and stability studies. Further, interaction studies were carried out. Bioavailability studies were performed for *Ficus religiosa* L. extract in suspension form and SLN form. AUC of lupeol was increased by 9.2 - folds in rats treated with SLN compared to suspension. Also, C_{max} of lupeol was increased by 3.9 - folds in rats treated with SLN compared to suspension. $t_{1/2}$ of lupeol was found to be 7.3 ± 1.0 h in suspension and 15.3 ± 1.3 h in SLN. SLN enhanced AUC and C_{max} and prolonged $t_{1/2}$ of lupeol in *Ficus religiosa* L. extract which in turn may lead to dose reduction, prolonged duration of action and also enhanced therapeutic efficacy.

1. Introduction

Herbal drugs as single phytochemical or as extracts are known for their mixed pharmacological actions with the benefit of no or less harmful side effects. Phytochemicals such as curcumin [1], berberine [2] and paclitaxel [3] are well known for their effective pharmacological actions without or with less side effects and extracts such as Artichoke leaf extract [4], *Cimicifuga foetida* extract [5], *Andrographis paniculata* extract [6] and *Ginkgo biloba* extract [7] are widely used in the treatment of various diseases. The availability of synthetic drugs is more in the market than herbal drugs. The presence of several phytochemicals in a single plant or extract causes difficulty in carrying out qualitative and quantitative analyses. For qualitative and quantitative analyses, there is a need of reference compounds for each phytochemical present which results in higher cost of the experiment. Also, due to unavailability of pharmacokinetic parameters of most of the phytochemicals in the current scenario limits the use of natural products.

Ficus religiosa L. possesses strong anti-oxidant activity and it is included in several ayurvedic formulations for the treatment of diabetes, epilepsy, inflammatory conditions, microbials, gout, stomatitis, leucorrhea, ulcers and against several microbes [8,9]. Phytoconstituents present in the bark, leaves, fruits, latex and decoction of *Ficus religiosa* L. are responsible for its pharmacological actions. Alcoholic extracts (ethanolic and methanolic extracts) of *Ficus religiosa* L. bark were reported to contain phytosterolins such as lupeol, β-sitosterol, β-sitosterol-d-glucoside, stigmasterol, lanosterol and campesterol. Octacosanol, methyl oleonate and lupen-3-one were reported in petroleum ether extract of the bark. In the benzene extract of bark, furanocoumarins (bergapten and bergaptol) were reported [10].

Even though several marketed ayurvedic formulations of *Ficus religiosa* L. are available, there is very less information about its qualitative and quantitative estimations and there is no reported pharmacokinetics data. In this context, we aimed to perform qualitative and quantitative estimations to find out the major

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compound present in *Ficus religiosa* L. extract and proceeded to estimate the bioavailability of the major compound and developed a drug delivery system which enhances the bioavailability of the major compound. Solid lipid nanoparticles (SLN) are well known for enhancing bioavailability of poorly water soluble drugs by lymphatic uptake [11].

In the present study, pharmacokinetic parameters of *Ficus religiosa* L. extract were estimated. To avoid the complexity associated with analyzing each phytochemical present in the extract, this study focused on major phytochemical present in the ethanolic extract of *Ficus religiosa* L. Further, *Ficus religiosa* L. extract loaded SLN were developed. Comparison of pharmacokinetic profile of *Ficus religiosa* L. extract suspension with developed *Ficus religiosa* L. extract loaded SLN has been carried out and found that the major compound was found to have increased bioavailability in SLN form. Briefly, *Ficus religiosa* L. extract loaded SLN were prepared by using two lipids separately (glyceryl monostearate and compritol ATO 888) by hot homogenization followed by ultrasonication method using binary surfactant mixture (poloxamer 188 and sodium deoxycholate in the ratio of 25:75). Developed *Ficus religiosa* L. extract loaded SLN were characterized by particle size and PDI, zeta potential, entrapment efficiency, *in vitro* drug release and kinetics, fourier transform infra-red spectroscopy (FTIR), differential scanning calorimetry (DSC), powder Xray diffractrometry (PXRD) and stability studies. Based on *in vitro* characterization, best formulation was selected and bioavailability studies were conducted for *Ficus religiosa* L. extract suspension and SLN. From the results, it was found that the major compound present in the *Ficus religiosa* L. has very less bioavailability and the poor bioavailability was tremendously increased by SLN. We strongly anticipate that this study may provide pharmacokinetics parameters of lupeol present in *Ficus religiosa* L. extract and its bioavailability enhancement through SLN.

2. Materials and methods

2.1. Materials

Glyceryl monostearate, poloxamer 188 and sodium deoxycholate were obtained as gift samples from Hospira Pvt. Ltd, Chennai, India. Dialysis membrane (cutoff MW 12,000–14,000 Da) was procured from HiMedia, India. Standard, lupeol (with the purity of 95% by HPLC) was procured from Sigma Aldrich, India. Water used in all experiments was purified by Milli-Q-plus system (Millipore, India). All other chemicals and solvents were of analytical grade.

2.2. Plant studied

Plant studied in this study was *Ficus religiosa* L. Local name of *Ficus religiosa* L. is peepal tree. The plant name has been checked with www.theplantlist.org mentioning the data of accessing that website. The stem barks of *Ficus religiosa* L. were collected in the month of November from the locality of Banaras Hindu University, Varanasi, India and authenticated by Prof. R. S. Upadhyay, Department of Botany, Banaras Hindu University, Varanasi, India.

2.3. Preparation of ethanolic extract of Ficus religiosa L.

Stem barks of *Ficus religiosa* L. were dried under sun for 15 days and powdered finely. 50 g of this powder was taken into the porous container of soxhlet apparatus. 500 ml of ethanol was used for extraction; 250 ml of ethanol was taken in a distilling pot and remaining ethanol was poured into porous container. Temperature of 40 °C was maintained and soxhleted for 48 h. Further, solvent was recovered and dried the extract. Traces of organic solvent were completely removed at 70 °C using rotary evaporator (IKA RV 10) by nitrogen gas purging. Dried ethanolic extract was used for the study.

2.4. Standardization of Ficus religiosa L. extract

For qualitative analysis, LCMS was carried out by using quadrapole time-of-flight spectrometer (G6520B, Agilent technologies) equipped with a electrospray ionisation source in both positive and negative modes. Minimum range and maximum range of 50 and 1500, respectively were used. Other parameters such as scan rate of 1, gas temperature of 300 °C, gas flow of 10.0 l/minute and nebulizer pressure of 32 psi were used. Thin layer chromatography (TLC) was performed by using Silicagel G as adsorbent and n-butanol: acetic acid: water (4:0.5:5) as mobile phase. Plate was prepared by pouring silica gel slurry on glass plate and activated by heating at 110 °C for 30 min. The spots were detected using vanillin reagent in sulphuric acid and R_f values were calculated. Preparative TLC using silica gel 60F254 precoated TLC plates (Merck) 20 × 20 cm was carried out by partitioning 25 g of ethanolic extract with 50 ml of petroleum ether. This extract was then concentrated and used for isolating major compound. FTIR analysis of major compound was done by using FTIR-8400S, Shimadzu by conventional KBr disc/pellet method. An FTIR spectrum was measured over the range of 4000–400 cm^{-1} with resolution of 4 cm^{-1} for 50 scans. Structural elucidation of major compound was obtained from ^{1}H NMR and ^{13}C NMR analyses by using Bruker 400 Avance Spectrometer operating at 400 MHz using tetramethylsilane as internal standard.

2.5. RP HPLC analysis

For the quantification of major compound present in *Ficus religiosa* L. extract, RP HPLC was carried out. RP HPLC setup (Waters, USA) comprising of binary pumps and PDA 2998 detector was used. Spherisorb ODS2 C18 column with dimensions of 250 × 4.6 mm was used. Acetonitrile: methanol: water combination was used as mobile phase in the ratio of 40:40:20. The mobile phase was filtered through 0.45 μm nylon filters (Millipore, USA). 20 μL volume samples were injected at the flow rate of 1 ml/min and analyzed at λ_{max} of 211 nm.

2.6. Preparation of Ficus religiosa L. extract loaded SLN

SLN were prepared by the combined method of hot homogenization and ultrasonication. Composition of all SLN formulations is shown in Table 1. Lipid (either glyceryl monosterate or compritol ATO 888) was melted at 5–10 °C above the melting point of lipid and *Ficus religiosa* L. extract was dispersed into the melted lipid phase. Aqueous phase containing 0.5% concentration of surfactant (combination of poloxamer 188 and sodium deoxycholate in the ratio of 25:75) was poured into the lipid phase which was maintained at the same temperature of lipid phase. Then, homogenized at 12,000 rpm for 30 min using high speed homogenizer (T25, UT, IKA). SLN suspension was then allowed to cool at room temperature. Sonication of formed suspension was performed using

Table 1
Composition of SLN formulations.

Ingredients	SLN 1	SLN 2	SLN 3	SLN 4	SLN 5	SLN 6
Drug (%)	0.5	0.5	0.5	0.5	0.5	0.5
Glycerylmonostearate (%)	1	2	3	–	–	–
Compritol ATO 888 (%)	–	–	–	1	2	3
Surfactant (%)	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water (ml)	50	50	50	50	50	50