SILYMARIN: REVIEW ON OVERALL DEVELOPED FORMULATIONS AND ANALYTICAL METHODS

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Abstract : Silymarin is flavonoid obtained from Silybum marianum plant. It is used as hepatoprotective, antioxidant, anti-inflammatory, hypolipidemic, and anti-carcinogenic properties[44]. Silymarin is BSC II class drug contains eight flavonolignans which having poor aqueous solubility due to this it rapidly excreted in bile and urine and poor bioavailability of these flavonolignans after oral route administration due to phase II metabolism, therefore in this review a potential approach to enhance solubility, bioavailability and to develop a robust formulation and scientific aspects from past to present is studied . In cancer treatment Nanomaterials are frequently used as drug carriers [12]. There is a need to have a selective and accurate analytical method for qualitative and quantitative estimation of silymarin flavonolignan elements[a]. Silymarin containing products have an important role in the quality control of milk thistle-based products. Due to the low concentrations of analytes, especially pharmacological and pharmacokinetic studies require more and more selective and sensitive, advanced techniques [N]. Therefore, the scope of this review was to develop a better understanding of the problems associated with silymarin and approaches to overcome the difficulties to develop a better and stable formulation for food and pharmaceutical applications.

KEY WORDS: silymarin, hyphenated techniques, formulation, pharmacological activity.

Introduction : Silymarin is commonly named as milk thistle which is chemically known as 2-(2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-1,4-benzodioxin-6-yl)-2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one relating to the family Asteraceae(1)

So for Silymarin is used as Antiflammatory activity, Hepatoprotective activity, Anticancer, Immunostimulatory, Antiasthmatic, neuroprotective, prevent the gastrointestinal problem, anti-diabetes, skin protection, modulate drug transport in multidrug resistance.[47] (2)Due to these pharmacological activity silymarin newly having attention. Therefore in this review, we have summarized silymarin formulation and analytical method for understanding & overcome problems associated with formulation hence in future we are able to develop stable formulation and its roust analytical method development (1)

Pharmacokinetics and bioavailability: [sylibin pdf]

Silymarin is hydrophobic & non-ionizable in nature, elimination half-life is 6 hours excreted in unchanged form in bile and urine .which having water solubility is less than 50µg/ml which has great influence on bioavailability which makes challenging in the formulation. After oral administration 240mg of silymarin rapidly absorb from stomach with Tmax about 1.8-1.9hour and t1/2 about 2.5-3.8 hours, C max 2.9± 0.3µg/ml,area under curve 10.8±0.4µg/ml ×h because of these problem silymarin need formulate in Nanomaterials or phytosomes having some advantages as drug carriers are increasing water solubility , dissolution of drug in bloodstream is protected, improving the pharmacokinetic and pharmacological properties and targeted drug delivery was achieved (3). (9,10)(60,61)

FORMULATIONS ASPECTS: Enhancement of solubility, bioavailability of drug is very challenging task of drug developments process.The solubility &dissolution property of drug playan important role in formulation.this problem of poor solubility &bioavailability is major challenge for formulation which can be solved by following some summarized formulation techniques

Formulation	Preparation method	Component of formulation	Research highlights	Reference
		TABLETS		
Fast dissolving tablet	Dry granulation method	Cross povidone, Microcrystalline Cellulose, Croscarmellose sodium,Aerosil	fast dissolving tablet (FDT) with improve patient compliance and convenience	Brij Mohan et . al (2014)
Microporous osmotic pump tablets		Dibutyl phthalate, soybean lecithin,sodium chloride, lactose, mannitol	Sustained and controlled-release drug delivery	Qi-ping Zeng et al (4)
Floating tablets	Wet granulation	HPMC, MC, crospovidone	Prolong gastric residence time	Garg and Gupta
Osmotic tablets	Melt fusion	Cellulose acetate	Controlled release of silymarine components	Xie et al (2013)
Erodible matrix tablets	Melt fusion	GMS,PEG-6000, Poloxamer-188	Controlled release of silymarine components	Lu et al. (2007)
Solid dispersion tablets	Direct compression	HP-β-CD	Enhance dissolution and oral bioavailability	Yanyu et al (2006a)
		EMULSION		
Emulsion	Membrane emulsification	Poly(lactic-co-glycolic acid) (PLGA), Polycaprolactone (PCL), Sodium Alginate, Chitosan, poly(Llactide) (PLLA), Eudragit,	Improve encapsulation efficiency and drug loading.	Emma Piacentini1 et al (2017)
Nano emulsion	Aqueous titration method	Sefsol 218 , KolliphorRH40 ,polyethylene glycol 400	efficient carrier for oral delivery of silymarin against human hepatocellular carcinoma without damaging normal	Usama Ahmad (2017)(5)
Nano emulsion	Spontaneous emulsification	Sefsol-218, tween-80, ethanol	Enhance bioavailability and hepatoprotective activity	Parveen et al. (2011a)(6)
		SUSPENSION		
Nano suspension	Micro emulsion dilution	BL, FL-40, Ethanol	Enhance stability	Hui and huihua (2011)
		INCLUSION COMPLES		
Inclusion complex	Kneading method	β-CD	Enhanced dissolution and bioavailability	Arcari et al(1992)
Inclusion complex	Kneading, co-precipitation and solvent evaporation	β-CD	Enhanced dissolution and solubility	Ghosh et al

Inclusion complex	physical mixing and kneading methods	Fulvic acid,	improve the solubility and dissolution profile	Shamama Javed et al (7)
Phospholipids complex	Solvent evaporation	phospholipid	Enhanced dissolution and bioavailability	
Phytosomal complex	-	phosphatidylcholine	Enhanced bioavailability	
		SOLID DISPERSION		
Solid dispersion	Fusion method	PEG-6000	Enhanced dissolution rate	Li et al (2002), Li and Hu (2004)
solid dispersion	spray drying and co-precipitation methods	HPMC E 15LV,	enhanced silymarin dissolution	DALWADI SONALI et al (8)
Solid dispersion	Fluid bed techniques	PVP	Enhanced dissolution rate	Sun et al (2008a)
Solid dispersion	Fluid bed techniques	PVP	Enhanced oral bioavailability	Sun et al (2008) (6)
Solid dispersion	Kneading, spray drying , co precipitation	HPMC	Enhanced dissolution rate	Sonali et al (2010)
solid dispersion	supercritical fluids method	PVP K30, HPMC K4M and HPMC K15M, Carbon dioxide	improve the dissolution and bioavailability	Gang Yang et al (9)
solid dispersion	spray drying and co-precipitation methods	HPMC E 15LV,	enhanced silymarin dissolution	DALWADI SONALI et al (8)
		SOLID- LIPID NANO-PARTICLES		
SLNs	High pressure homogenization	-	Enhanced biodistribution	Parveen et al(2011a,(6)
SLNs	Lyophilization	Compritol -888ATO, lecithin, Poloxamer-188	Enhanced biodistribution and bioavailability	He et al(2005,2007)
SLNs	High pressure homogenization	Brij 87, stearic acid	Controlled release of silibinin	Zhang et al (2007)
SLNs	Cold homogenization	Compritol -888ATO, poloxamer-188	Physicochemical characterization	Raffa et al (2010)
		NANO-PARTICLES		
PLGA nanoparticles	modified emulsification/ solvent evaporation method	Poly(D,L-lactide-co-glycolide),	improve the overall entrapment efficiency and to reduce the escaping ratio	Yunchang Xie Et al (10)
Solid Lipid Nanoparticles	-	-	improve the stability and capacity loading.	Neda Naseri Et al (11)
Eudragit loaded nanoparticles	nanoprecipitation technique	Eudragit RS100 Eudragit RL100, Polyvinyl alcohol, Hydroxypropyl methyl cellulose	to improve the low bioavailability of silymarin through buccal delivery.	Amira E. El-Nahas et al (12)
porous silica nanoparticles	microemulsion and ultrasonic corrosion methods	Octylphenol polyoxyethylene, cyclohexane, a-naphthol	improve oral bioavailability	Xia Cao et al (13)

		NANOCRYSTAL		
Nanocrystals	high pressure crystallizer	hydroxypropyl-β-CyD	enhanced dissolution rate and absorbability	Risako Onodera et al (14)
		NANOSTRUCTURES LIPID CARRIER		
Nanostructured lipid carriers		Glycerol monostearate Oleic acid Tween 80	*In vivo* study revealed high accumulation of drug in liver after encapsulation in NLCs	Chaudhary S,
Binary lipids-based nanostructured lipid Carriers	high-pressure homogenization	Oleic acid ,Tween-80,	improve the oral bioavailability of silymarin	Mingzhu Shangguan Et al(13)
Topical nanostructured lipid carrier	Hot high-pressure homogenization process.	Glycerolmon,Oleic Acid,carbopol 980	enhanced solubility and stability of silymarin and greater permeation into the affected cell	Pooja Singh et al (15)
Nanostructured lipid carriers:	Hot high-pressure homogenization process.	Compritol ATO 888, Pluronic F-68	increase the therapeutic value by better permeation, anticancer action and reduced toxicity	Pooja Singh et al (16)
		LIPOSOMES		
Liposome	supercritical fluid technology	Soybean phosphatidylcholine, SGC,	to improve the dissolution and bioavailability of silymarin	Gang Yang et al
Liposome	Ethanol injection	Cholesterol	Ehnaced hepatoprotective and gastroprotective effect	Maheshwari et al (2003)
Liposome	Reversed evaporation techniques	Lecithin, cholesterol, stearyl amine, tween-80.	Ehnaced hepatoprotective effect.	EI- Samligy et al (2006)
Liposome	-	*ρ*-amino phenyl- *β*-D-Galactopyranoside	Targetting to hepatocyte	Dube et al (2010)
Liposome	Film hydration	Lecithin, cholesterol e	Targetting to hepatocyte and enhanced bioavailability	Kumar et al (2014)
		PROLIPOSOMES		
Proliposome	simple dissolving process	Soy-lecithin, DGalactosamine,SOD, MDA, GSH-PX	To improving silymarin's poor bioavailability and hepatoprotective effects	Mei Wang et al
Proliposomes	Film deposition	Phospholipid and mannitol	Enhanced bioavilability	Xiao et al (2005) and Yanyu et al

				(2006)
Nanoliposomes	extrusion method.	Cholesterol,egg lecithin, dioxane,SCDA	to evaluate efficacy of silymarin in free and nanoliposomal forms	Zohreh Faezizadeh et al
Bilosomes	film hydration technique	Soybean-lecithin phosphatidylcholine, Carbon tetrachloride	To increasing the hepatoprotective activity of the drug.	A.M.Mohsen et al
		DENDRIMERS		
Dendrimer	Co-precipitation	EGCG, DMF, DCM, TFA	To enhance skin penetration and deposition	P. K. Shetty et al (2016) (17)
Dendrimer	Solvent evaporation method	PAMAM-G4 , PEG, TEA, THF, DMSO	To deliver the poorly soluble drug silybin	Carola Diaz, et al (2017)
Carbon Nanotubes	non-covalent approach	Polysorbate20, Polysorbate80, Polyethylene glycol, Chitosan,	prolonged and sustained release of SB	Julia Meihua Tan(18)
MICROPARTICLES				
Micoparticle	Low energy emulsification techniques	Lecithin, tween-20, tween-80, span-20, propylene glycol	Enhanced dissolution, therapeutic efficiency and bioavailability	Abrol et al (2004,2005)
Floating microsphere	Wet granulation	HPMC, MC, crosspovidone	Prolong gastric residence time	Garg and Gupta (2009)

ANALYTICAL PERSPECTIVE:

Separation of key components of silymarin is achieved by various techniques of analysis .many hyphenated techniques comes into existing which lead to successful identification of each components which are summarized below

Analytical method	Mobile phase /solvent	Wavelength (nm)/Other parameters	Purpose	Reference
1)Kinetic spectrophotometry	Potassium permanganate	530	Develop kinetic spectrophotometric method Rahman (et al. 2004)	Rahman (et al. 2004) (19)
2)UV–visible spectrophotometry	Methanol	287	Develop and validate spectrophotometric method	Meghreji et al. (2010) [a]
3)UV spectrophotometry	2,4-dinitrophenylhydrazine	490	Develop spectrophotometric method	marianumDezs˝o Csupor (2016) (20)
4)TLC	Chloroform-acetone-formi acid (75:16.5:8.5)	365	Develop TLC method	Wagner et al. (2006)
5)TLC	Formicacid :acetone:methylene chloride (8.5:16.5:75)	296	Develop stability-indicating TLC method	Ph. Eur. (2004)
6)HPTLC	Chloroform:acetone: Formic acid (9:2:1)	Wavelength-296 M.P- Chloroform–acetone–formic acid (9 : 2 : 1 v/v/v)	Develop stability-indicating HPTLC method	Parveen et al. (2010) (21)
7)HPLC	Water: acetonitrile:methanol at different proportion	Wavelength-289	Develop stability-indicating HPLC method	Quaglia et al. (1999) (6)
8)HPLC	KH2PO4:methanol: acetonitrile at different proportions	288	Develop stability-indicating HPLC method	Korany et al. (2013) [1.s2.0s1]
9)HPLC	Methanol:NaH2PO4 (45:55)	288	Develop and validate HPLC method	Hadad et al. (2009)
10)HPLC	Methanol:water:acetic acid at different proportion	280	Develop stability-indicating HPLC method	Cai et al. (2009) (22)
11)HPLC	water and methanol	Wavelength-290 Column- C18 Temp.- 40°C Flow rate - 0.75 ml/minute	Develop and validate HPLC method	Sunny Wallace(2003) (23)

12)HPLC	(a) 0.1 % formic acid in water and (b) 0.1 % formic acid in 80 % aqueous methanol	Wavelength- 288 nm Column C_{18}	Develop validate and optimized method	Elizabeth Mudge(2015) (24)
13)HPLC	methanol and solvent mixture (water: dioxane=9:1)	wavelength: 288 nm column C_{18} flow rate: 1.5 ml/min; column temp.: 40°C;	Develop method for determination of components	Tian-ming Ding(2001) (25)
14)HPLC –CE	Solvent A_Water acidified at pH 2.3 with 10% H3PO4 solution; Solvent B_ acetonitrile; Solvent C_methanol.	Wavelenght -289 nm Column C_{18} Flow rate -1 ml /min	Develop method for determination of components	M.G. Quaglia (1999) (6)
15)HPLC-HPCE	100 mM boric acid until pH 9 and added with 15% methanol, used as background electrolyte	Wenthength-200 nm. capillary having a total length of 43 cm	qualitative and quantitative data obtained by HPLC and HPCE were compared.	M.G. Quaglia (1999) (6)
16)HPLC –MS	Formic acid:methanol at different proportions	225 and 288	Develop HPLC–MS method	Kuki et al. (2012) (26)
17)UHPLC	0.1%formic acid in MeOH (B) and 0.1% formic acid in H2O (A),	Wavelenght- 288 Column-C18 Flow rate-0.5 mL/min Temp.-50˚C	Develop method for determination of components	Tyler N. Grafa(2016) (27)
19)UPLC–MS	Mobile phaseAwas water containing 0.1% formic acid. phase B was MeOH containing 0.1% formic acid.	Wavelenght 288 Coloumn - UPLC C18 flow rate - 0.25 mL/min.	Develop rapid UPLC-MS method	Wang et al. (2010) (28)
20)LC-MS	formic acid (A) and methanol/water/formic acid (90:10:0.1, v/v/v) (B)	Column-C18 flow rate of 0.2 mL/min Temp.40˚C	Develop sensitive method for the simultaneous	James I. Lee(2007) (29)
21) GC-MS	Carrier gas-Helium	Column-capillary flow rate-1.5 ml/min. Temp.-270˚C	Develop method for determination of components	D.IONESCU(2017) [T]

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