Nanocarriers: successful tools to increase solubility, stability and bioefficacy of natural products

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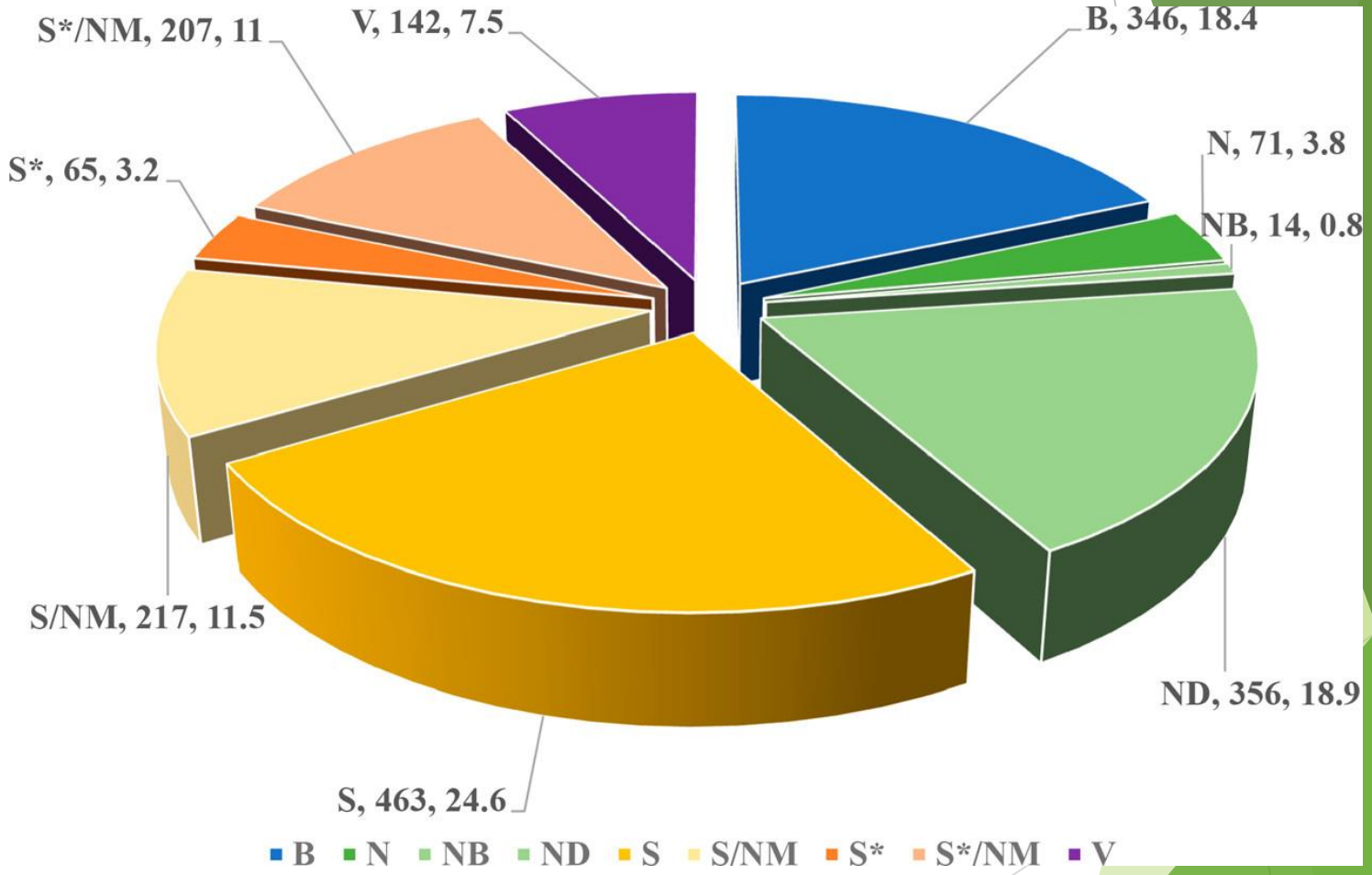
University of Florence

Nano 2020 Innovation
Rome, 15-18 September
Conference & Exhibition

YoungInnovation: the state of research communicated by young researchers – Nanotechnologies meet natural products

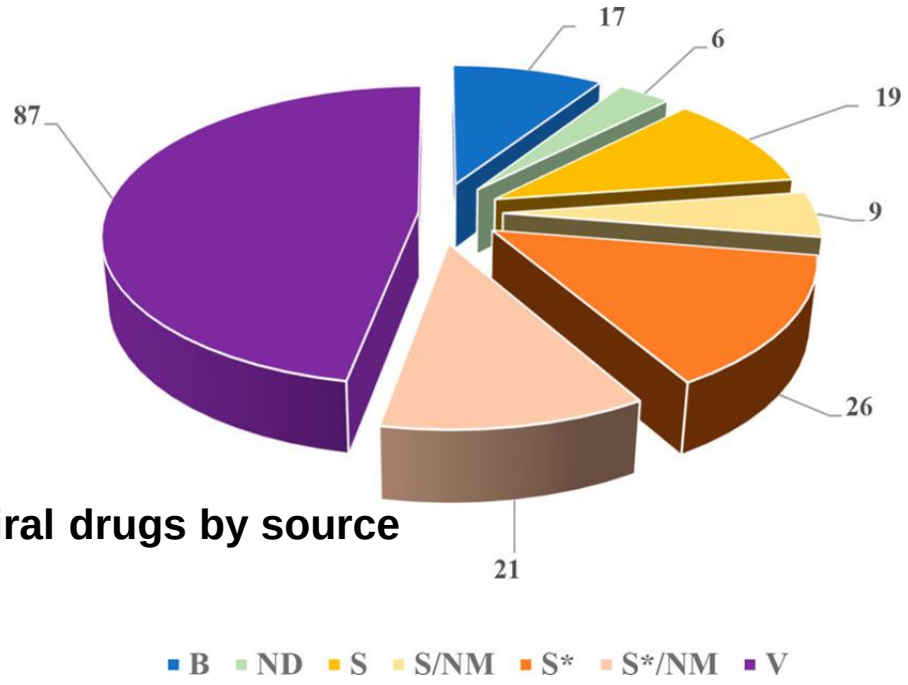
THE ROLE OF NATURAL PRODUCTS IN MEDICINE DURING THE LAST FORTY YEARS

code	brief definition
B	biological macromolecule
N	**unaltered natural product**
NB	**botanical drug (defined mixture)**
ND	**natural product derivative**
S	synthetic drug
S*	**synthetic drug (NP pharmacophore)**
V	vaccine
/NM	**mimic of natural product**

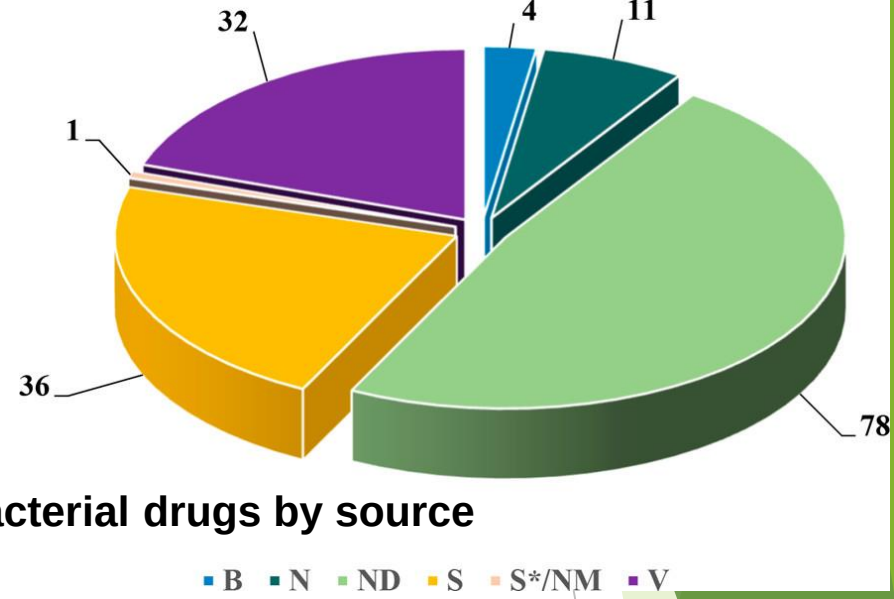


All new approved drugs 01JAN81 to 30SEP19; *n* = 1881.

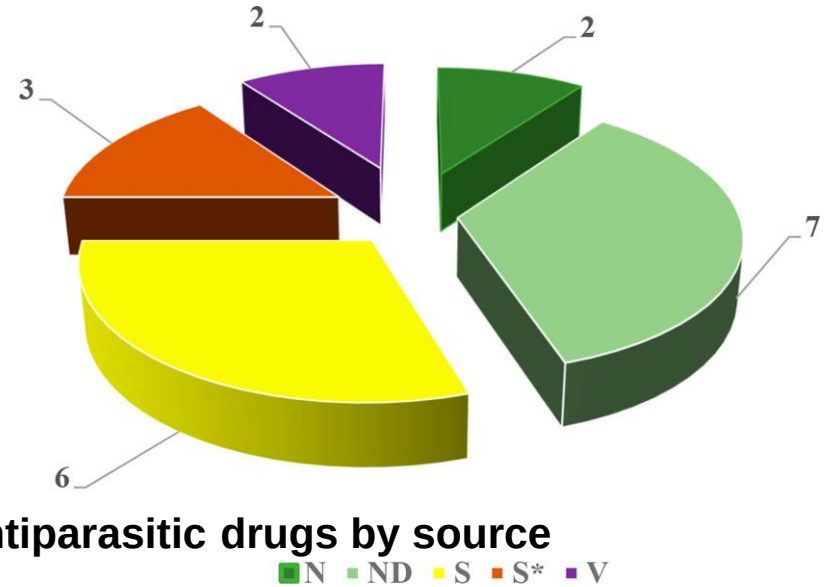
Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod. 2020 Mar 27;83(3):770-803. doi: 10.1021/acs.jnatprod.9b01285. Epub 2020 Mar 12.



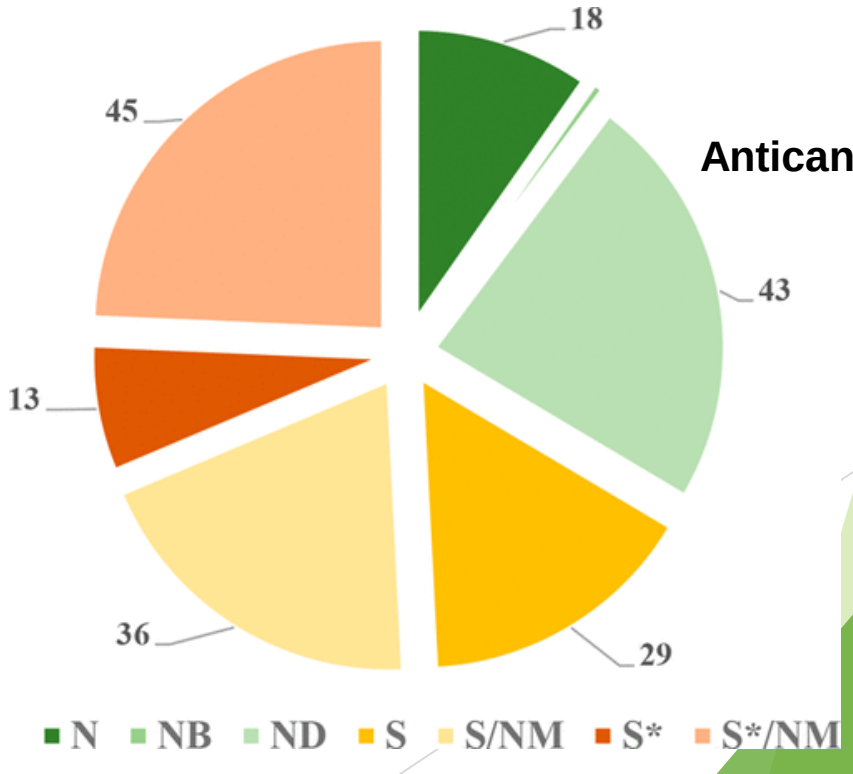
Antiviral drugs by source



Antibacterial drugs by source



Antiparasitic drugs by source



Anticancer drugs by source

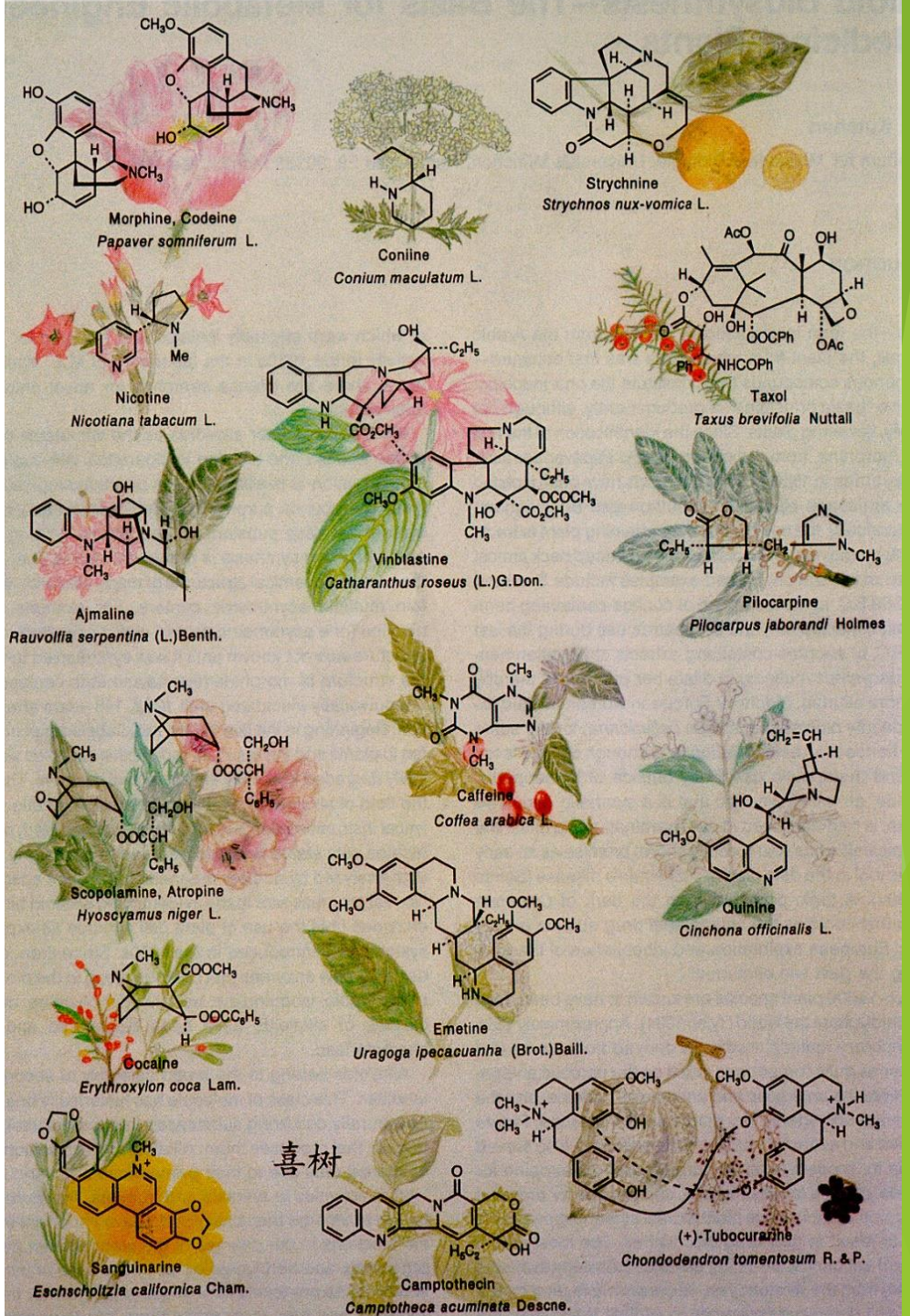
NATURAL PRODUCTS: about 326,000 molecules

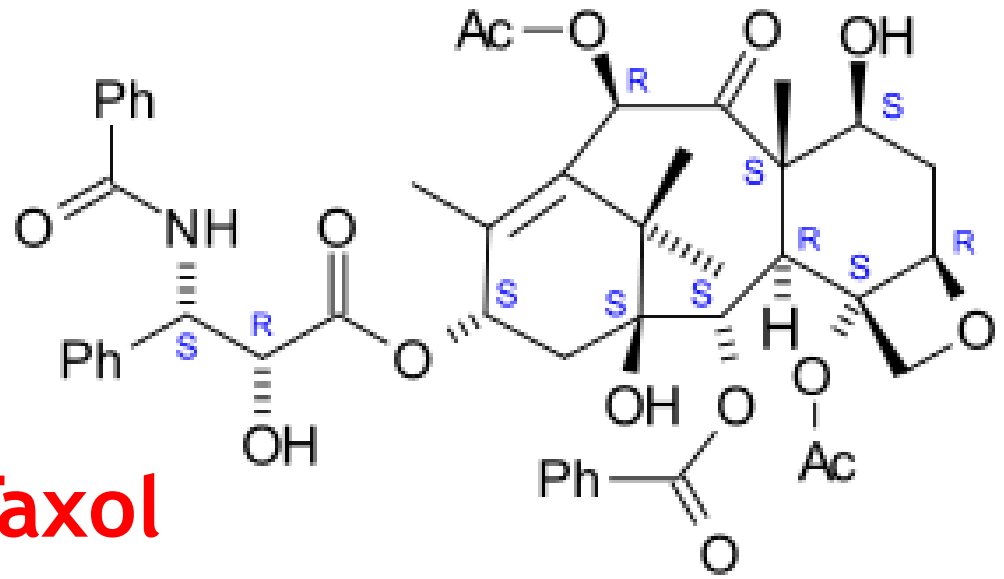
http://bioinformatics.charite.de/supernatural

http://dnp.chemnetbase.com/faces/chemical/ChemicalSearch.xhtml

WHY THERE IS AN INCREASING INTEREST IN NATURAL PRODUCTS?

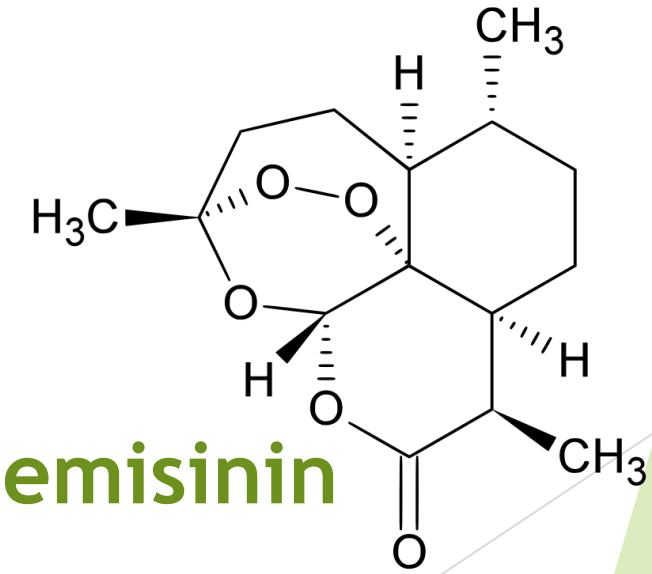
-unique structures and unique mechanisms of action





Taxol

Tubulin-targeting drug: taxol stabilizes the microtubule polymer and protects it from disassembly



Artemisinin

Activity: heme-mediated decomposition of the endoperoxide bridge to produce carbon-centred free radicals

NATURAL PRODUCTS: about 326,000 molecules

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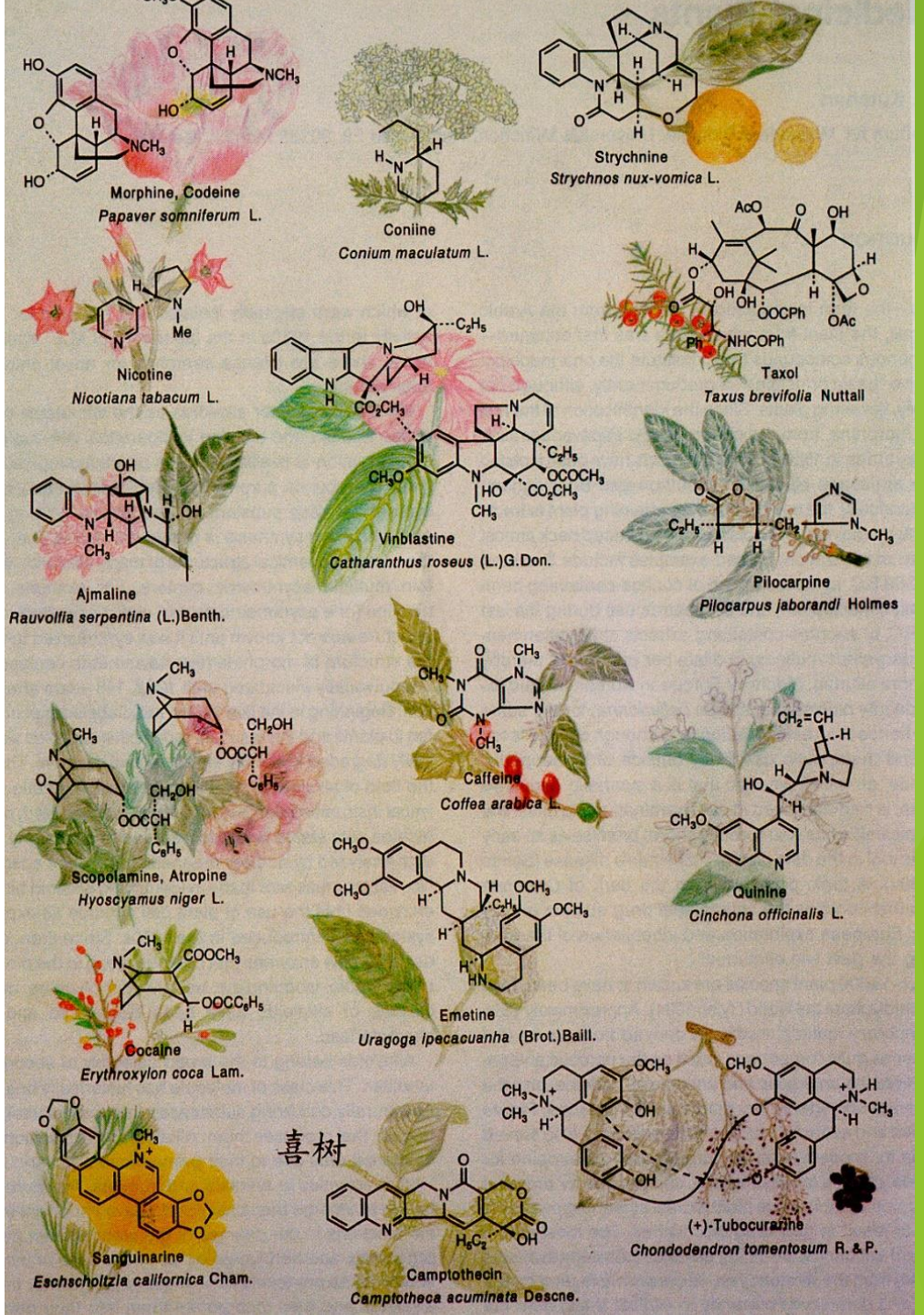
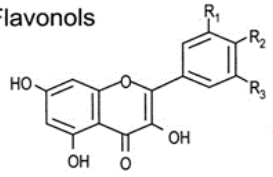
WHY THERE IS AN INCREASING INTEREST IN NATURAL PRODUCTS?

-unique structures and unique mechanisms of action

-beneficial health effects

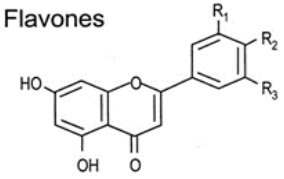


Flavonols



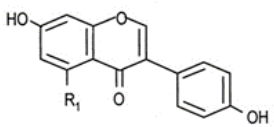
$R_2 = OH; R_1 = R_3 = H$: Kaempferol
$R_1 = R_2 = OH; R_3 = H$: Quercetin
$R_1 = R_2 = R_3 = OH$: Myricetin

Flavones



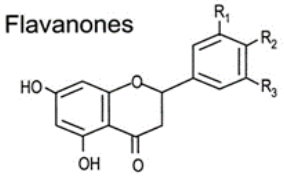
$R_1 = H; R_2 = OH$: Apigenin
$R_1 = R_2 = OH$: Luteolin

Isoflavones



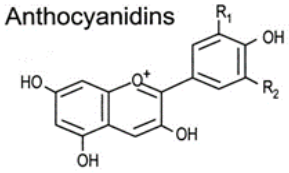
$R_1 = H$: Daidzein
$R_1 = OH$: Genistein

Flavanones



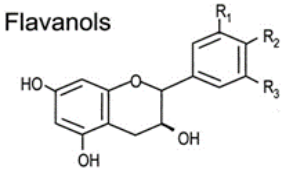
$R_1 = H; R_2 = OH$: Naringenin
$R_1 = R_2 = OH$: Eriodictyol
$R_1 = OH; R_2 = OCH_3$: Hesperetin

Anthocyanidins

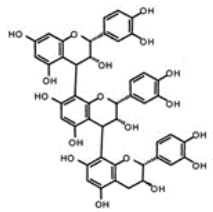


$R_1 = R_2 = H$: Pelargonidin
$R_1 = OH; R_2 = H$: Cyanidin
$R_1 = R_2 = OH$: Delphinidin
$R_1 = OCH_3; R_2 = OH$: Petunidin
$R_1 = R_2 = OCH_3$: Malvidin

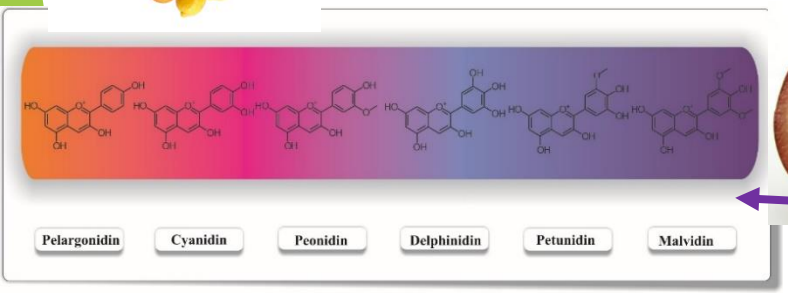
Flavanols

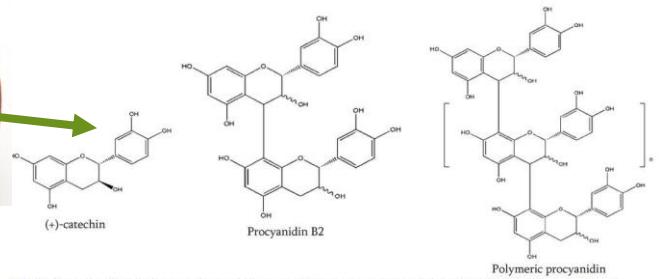


$R_1 = R_2 = OH; R_3 = H$: Catechins
$R_1 = R_2 = R_3 = OH$: Gallocatechin

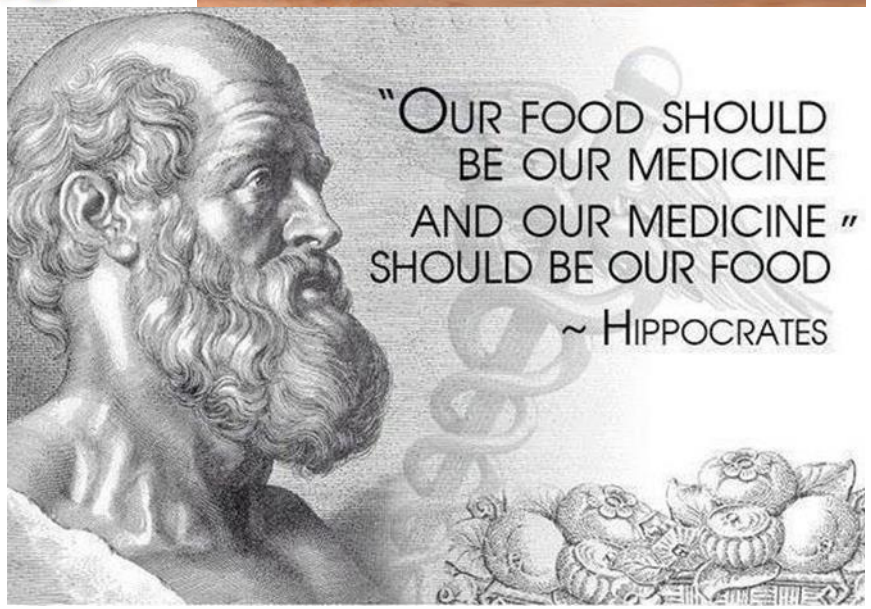


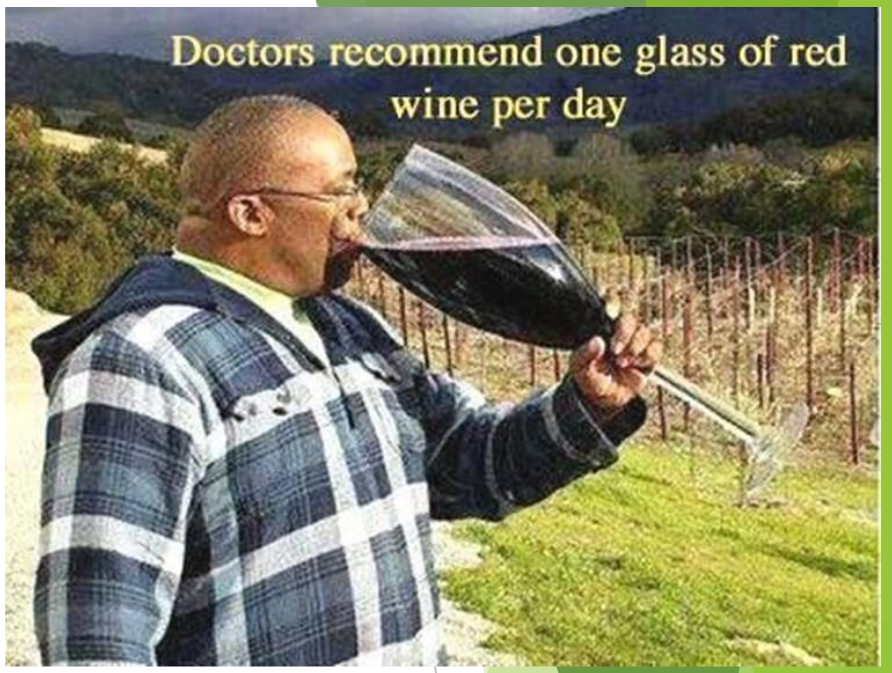
Trimeric procyanidin





(Modified from: Terra X, et al. Grape-seed procyanidins act as antiinflammatory agents in endotoxin-stimulated RAW 264.7 macrophages by inhibiting NFkB signaling pathway. J Agric Food Chem. (2007))





HOW MUCH RESVERATROL IN RED WINE?

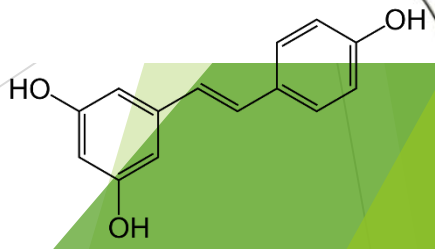
PINOT NOIR
California
5.01 mg/litre

BEAUJOLAIS
France
3.55 mg/litre

ZINFANDEL
California
1.38 mg/litre

CABERNET SAUVIGNON & MERLOT
Chile
1.56 mg/litre

CABERNET SAUVIGNON
California
0.99 mg/litre



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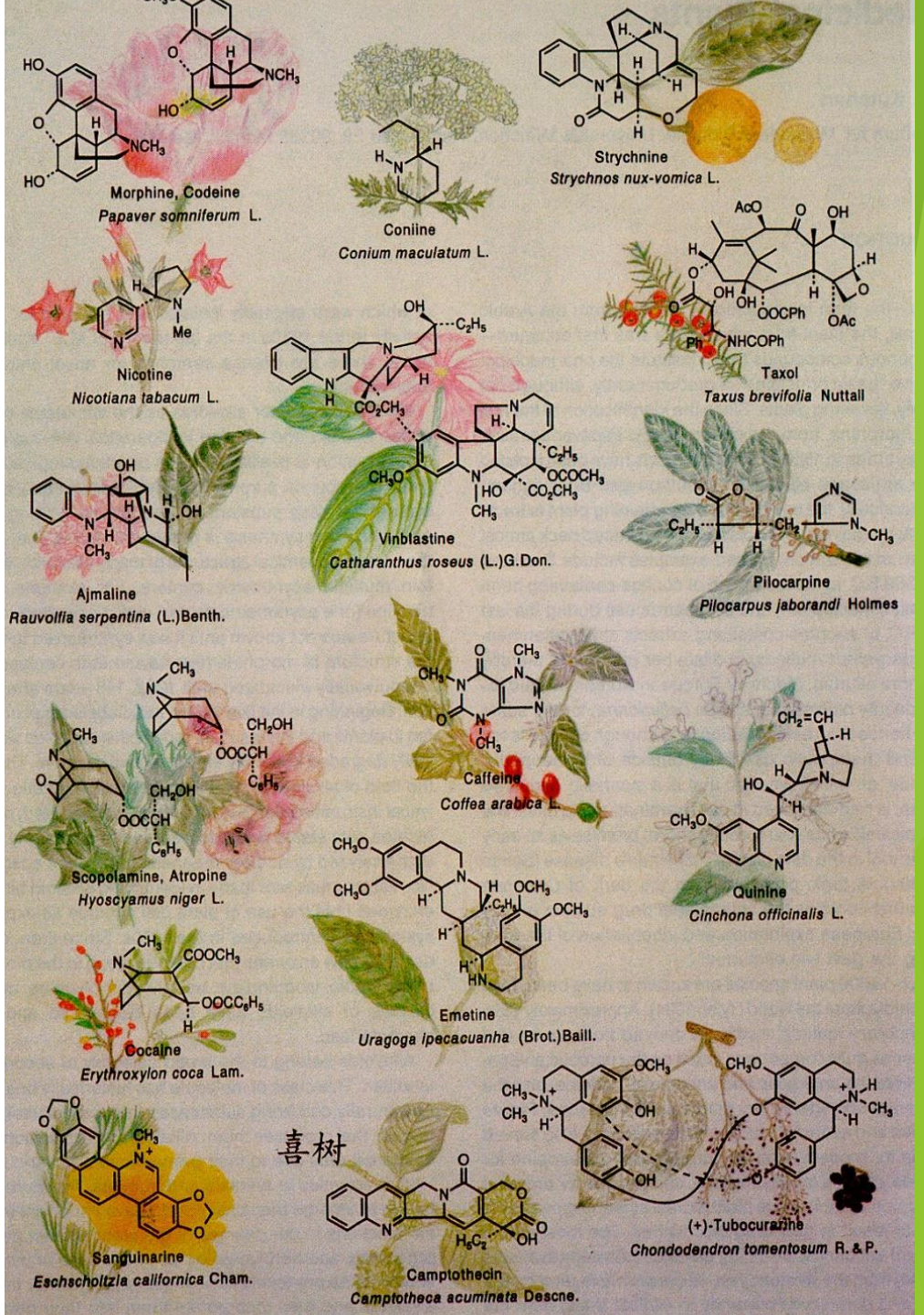
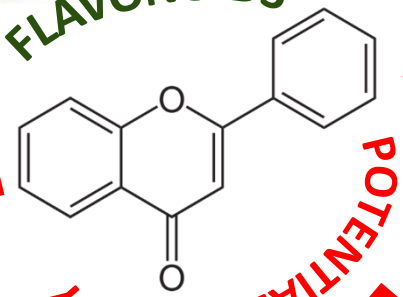
-unique structures and unique mechanisms of action
-beneficial health effects
-efficacious drugs
-safe and no side-effects associated



BIOCHEMICAL MECHANISMS

- ↑AMPK phosphorylation
- ↑PGC-1α expression
- ↓TLR4 expression
- ↓NF-κB activation
- ↑PKA activation
- ↓ERK1/2 phosphorylation
- ↓P38MAPK phosphorylation
- ↓MAPKs phosphorylation
- ↑PPAR-α expression
- ↑PPAR-γ expression
- ↑IRS2 expression and signalling
- ↑L-type calcium channels
- ↓SREBP-1c level
- ↓TNF-α level
- ↓tyrosine kinase

FLAVONOIDS



POTENTIAL ACTIVITY

SKELETAL MUSCLE/ CELLS
- ↑glucose uptake and oxidation
- ↑glucose tolerance and insulin sensitivity
- ↑mitochondrial biogenesis
- ↑adiponectin receptor 1 level
- ↓TG accumulation

MIOCARDIAL, RENAL TISSUE AND IMMUNE SYSTEM
- ↓pro-inflammatory citokines
- ↓inflammatory cell infiltra
- ↓apoptosis

BRAIN
- ↑nitric oxide levels
- ↓glucose transporters
- ↓ROS production
- ↓Beta secretase-1
- ↓acetylcholine
- ↑cholinesterases
- ↑ microglial activation
- ↓dopamine levels
- ↑ monoamino oxidase

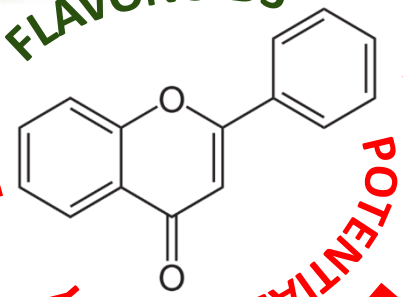
VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS
- ↑eNOS activation
- ↑nitric oxide levels
- ↑vasodilatation
- ↓blood pressure
- ↓ROS production

LIVER AND PANCREAS
- ↑β-cell survival and function
- ↑adiponectin receptor 1 and 2
- ↑fatty acid oxidation
- ↓lipogenesis
- ↓hepatic steatosis

ADIPOSE TISSUE
- ↑insulin-stimulated glucose uptake
- ↑adiponectin
- ↑GLUT4 gene expression
- ↑energy expenditure
- ↓inflammation
- ↑thermogenic gene expression in brown adipose tissue

The ability of NP to influence numerous biochemical and molecular cascades, could represent a realistic approach to many diseases, especially those with emerging resistance to monofunctional agents, and they are suitable approaches against multifactorial and complex diseases, especially cancer and diabetes.

FLAVONOIDS



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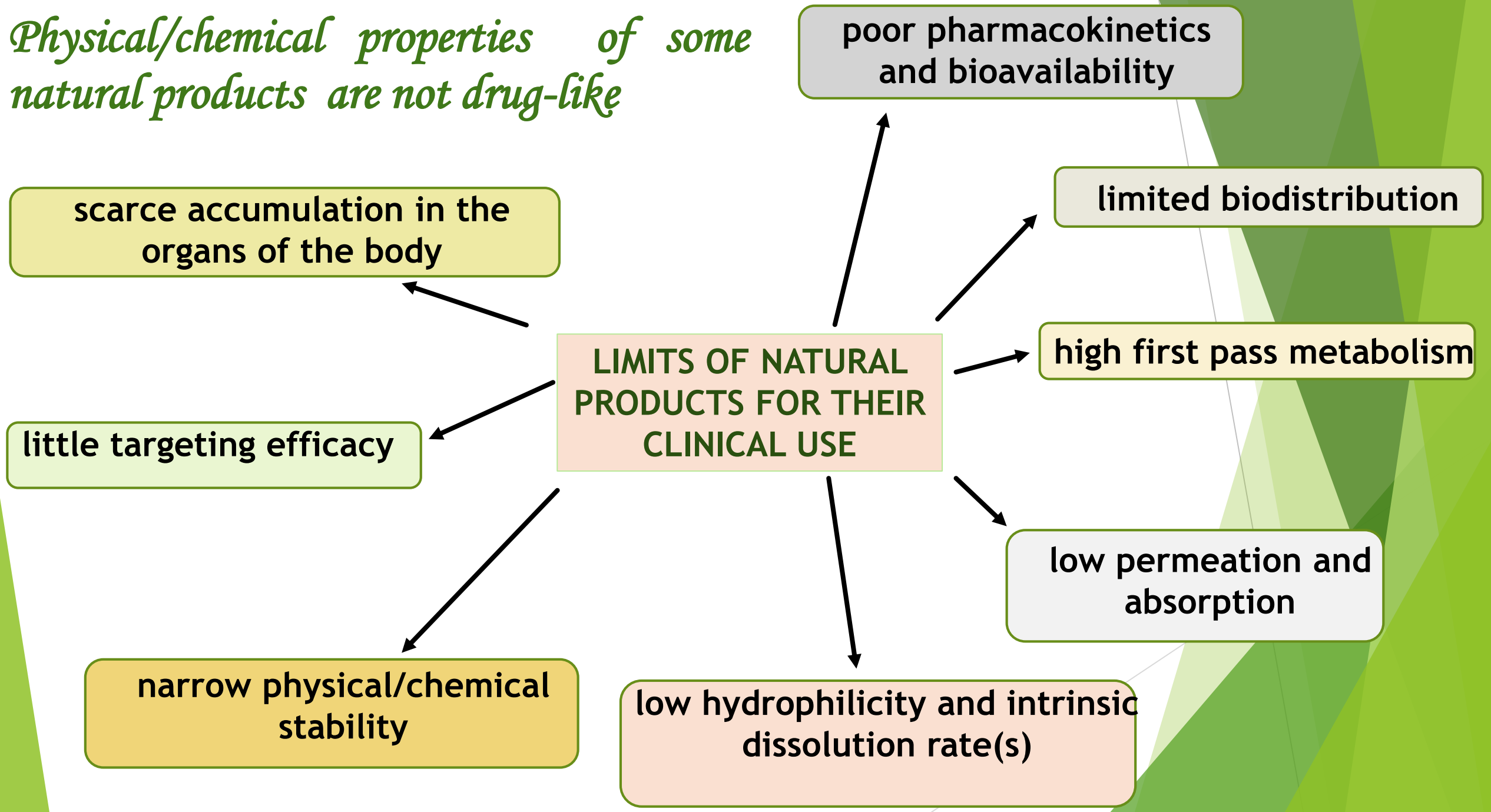
Physical/chemical properties of some natural products are not drug-like



NANOCARRIERS

- To optimize biopharmaceutical properties
- To obtain passive or active targeting
- To decrease doses and side effects
- To reach therapeutic levels over extended times

Bilia, et al. Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evid Based Complement Alternat Med. 2014;2014:651593. doi: 10.1155/2014/651593.

Bilia,et al. Flavonoids loaded in nanocarriers: an opportunity to increase oral bioavailability and bioefficacy. Food and Nutrition Sciences, 2014, 5:13, Article ID:47717,16 pages

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Bilia, et al. Plants Extracts Loaded in Nanocarriers: An Emergent Formulating Approach. Nat Prod Commun, 2018, 13 (9): 1157-1160.

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Efferth, et al. Expanding the Therapeutic Spectrum of Artemisinin: Activity Against Infectious Diseases Beyond Malaria and Novel Pharmaceutical Developments. World J Tradit Chin Med 2016; 2(2):1–23. DOI: 10.15806/j.issn.2311-8571.2016.0002

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Bilia. (2018) Use of nanocarriers to enhance artemisinin activity, Chapter 12, pages 271-295. In Artemisia annua: Prospects, Applications and Therapeutic Uses, edited by Tariq Aftab, M. Naeem, M. Masroor A. Khan. Taylor & Francis Group.

Bilia, et al. (2020) Nanotechnology Applications for Natural Products Delivery. In: Saneja A., Panda A., Lichtfouse E. (eds) Sustainable Agriculture Reviews 44. Sustainable Agriculture Reviews, vol 44. Springer, Cham. Chapter DOI: 10.1007/978-3-030-41842-7_1

International Journal of Pharmaceutics 416 (2011) 433–442

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Improving solubility and chemical stability of natural compounds for medicinal use by incorporation into liposomes

Maria Coimbra[a], Benedetta Isacchi[b], Louis van Bloois[a], Javier Sastre Torano[c], Aldo Ket[a], Xiaojie Wu[a], Femke Broere[d], Josbert M. Metselaar[a], Cristianne J.F. Rijcken[a], Gert Storm[a], Rita Bilia[b], Raymond M. Schiffelers[a,*]

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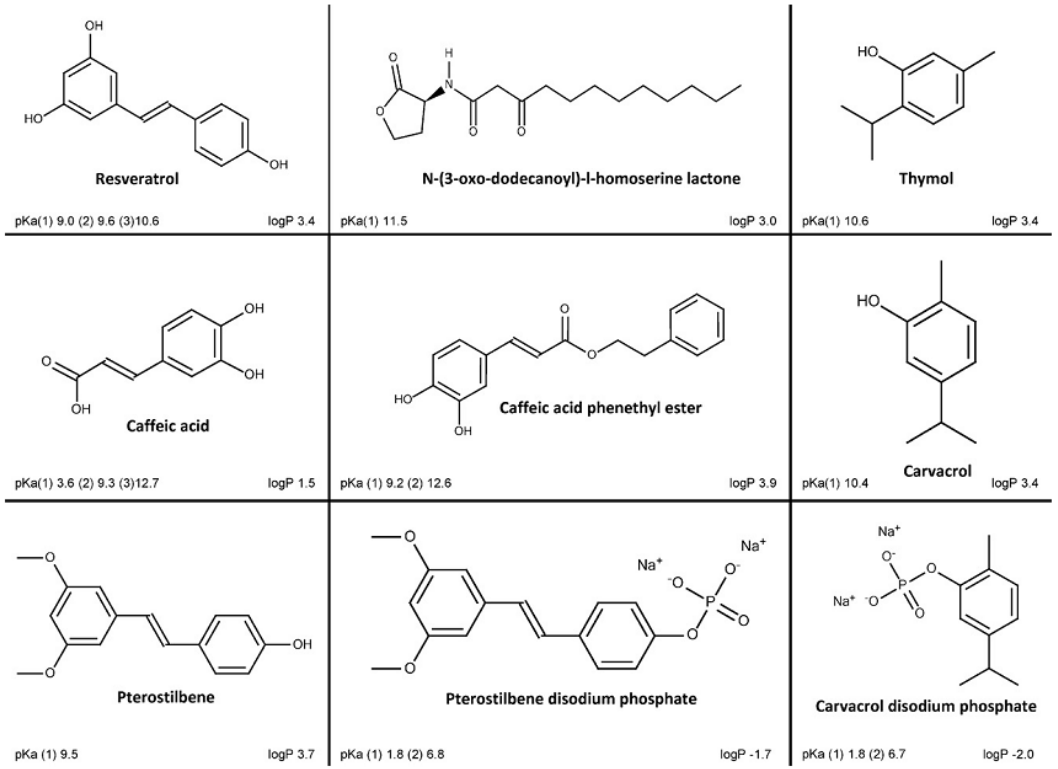
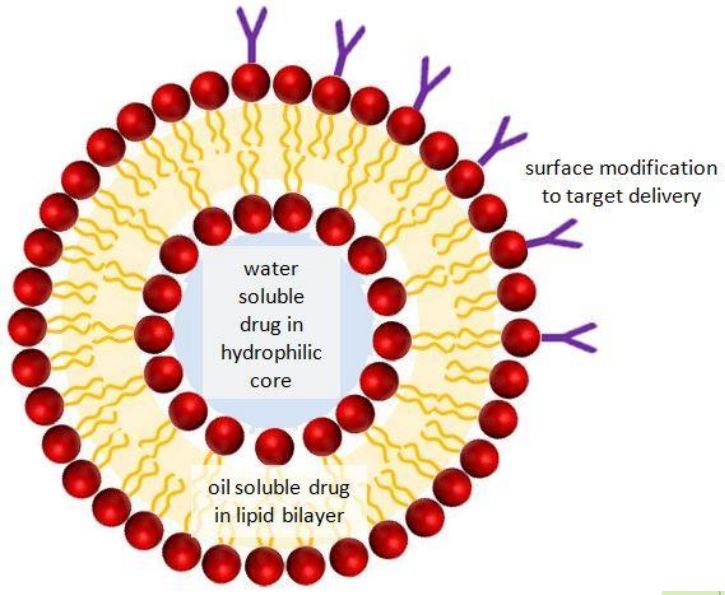
[b] Department of Pharmaceutical Sciences, University of Florence, via U. Schiff 6, 50019 Sesto Fiorentino, (FI), Italy
[c] Biomedical Analysis, Utrecht Institute for Pharmaceutical Sciences, Dept. of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
[d] Division of Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands



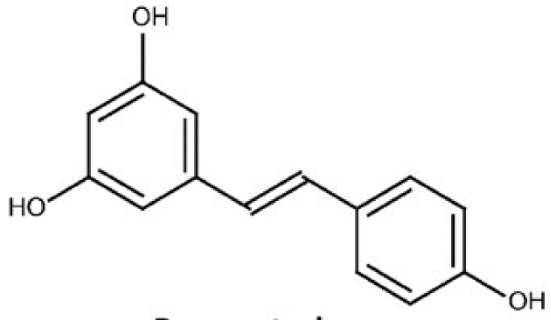
LIPOSOMES



CONCLUSIONS

Taken together, these results show that many poorly soluble natural compounds can be incorporated into liposomes. Compounds that are in the bilayer tend to be extracted in the presence of albumin, limiting the function of the liposome to that of a solubilizing excipient, simply allowing drug delivery. So far, the in vivo studies show that this may still be a valuable approach to obtain therapeutic benefits. However, if drugs can be stably encapsulated into long circulating liposomes, a targeted drug delivery can take place, which may further enhance biological activities of the compounds.

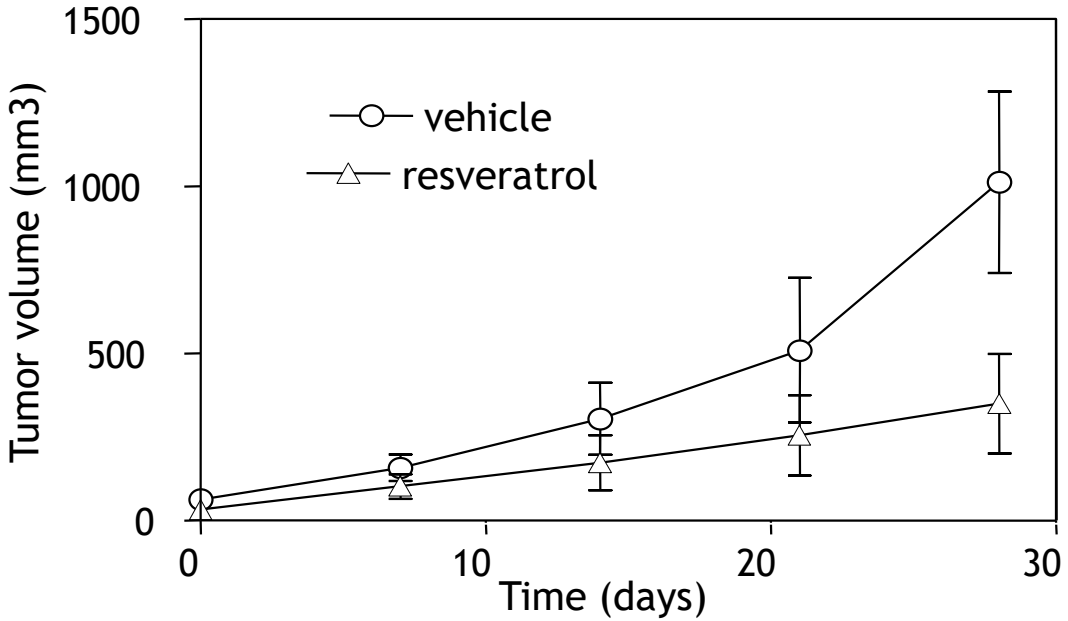
RESVERATROL encapsulated in long circulating liposomes (LCL)



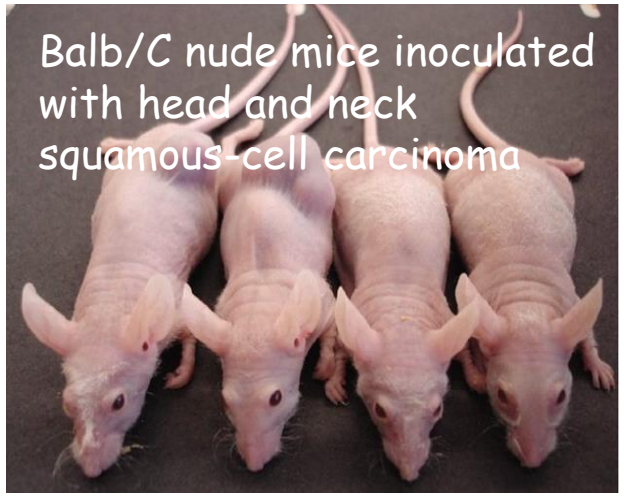
Resveratrol

Resveratrol exhibits a strong antioxidant activity and inhibition of hydro peroxidase, protein kinase C, Bcl-2 phosphorylation, Akt, focal adhesion kinase, NF-B, and matrix metalloprotease-9 (Kraft et al., 2009).

When tumours reached 50–100 mm^3, mice were included in the study, consisting of 6 mice per treatment group. At this time, mice received 5mg/kg liposomal resveratrol or equivalent dose of empty liposomes intravenously via the tail vein. Injections were repeated each 3 days



Coimbra et al., Int. J. Pharm. 416 (2011) 433- 442



vehicle

resveratrol-loaded LCL

RESULTS: Liposomes

-protect trans–cis isomerization
-protect under UV light exposure
-change bioavailability giving an effective antitumor response

European Journal of Pharmaceutical Sciences 44 (2011) 552–558

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European Journal of Pharmaceutical Sciences

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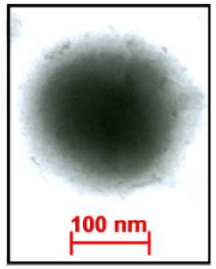
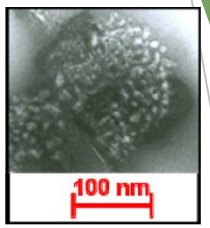


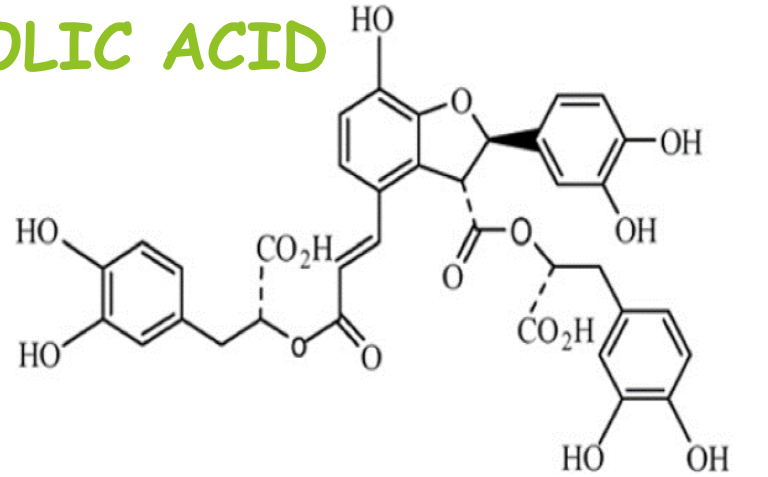


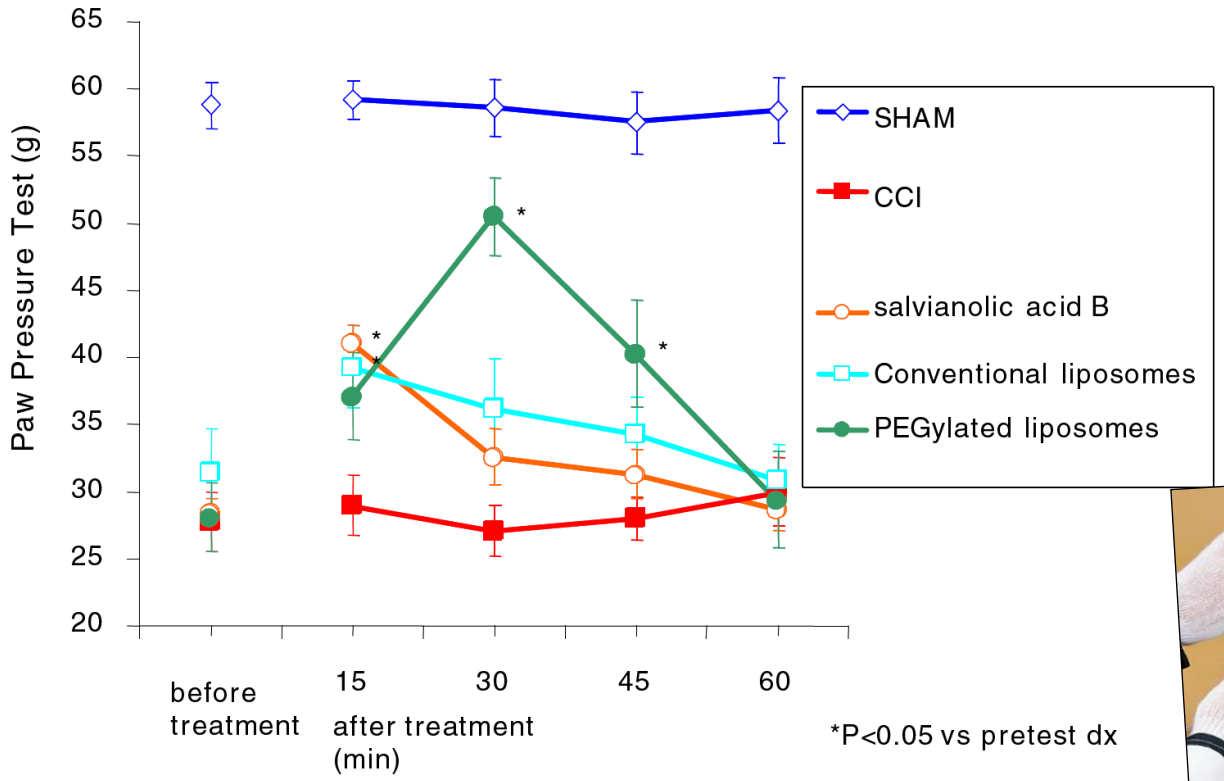
Salvianolic acid B and its liposomal formulations: Anti-hyperalgesic activity in the treatment of neuropathic pain

Benedetta Isacchi [a,*], Valentii
Carla Ghelardini [b], Maria Giu

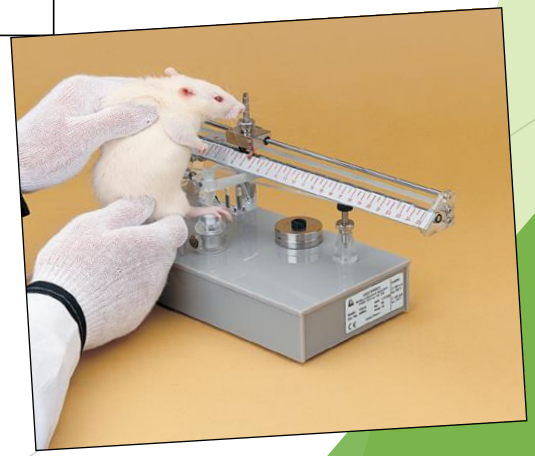
[a] Department of Pharmaceutical Sciences, Univer.
[b] Department of Preclinical and Clinical Pharmac
[c] Department of Anatomy, Histology and Forensi

SALVIANOLIC ACID





*P<0.05 vs pretest dx



SalB-loaded liposomes intraperitoneal administration in a chronic constriction injury of the sciatic nerve (CCI) in the rat paw-pressure test.

European Journal of Pharmaceutical Sciences 44 (2011) 552–558

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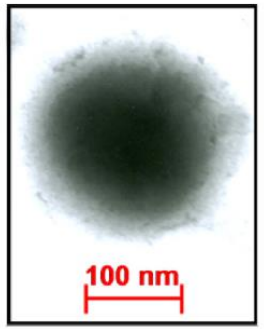
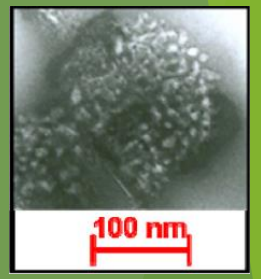
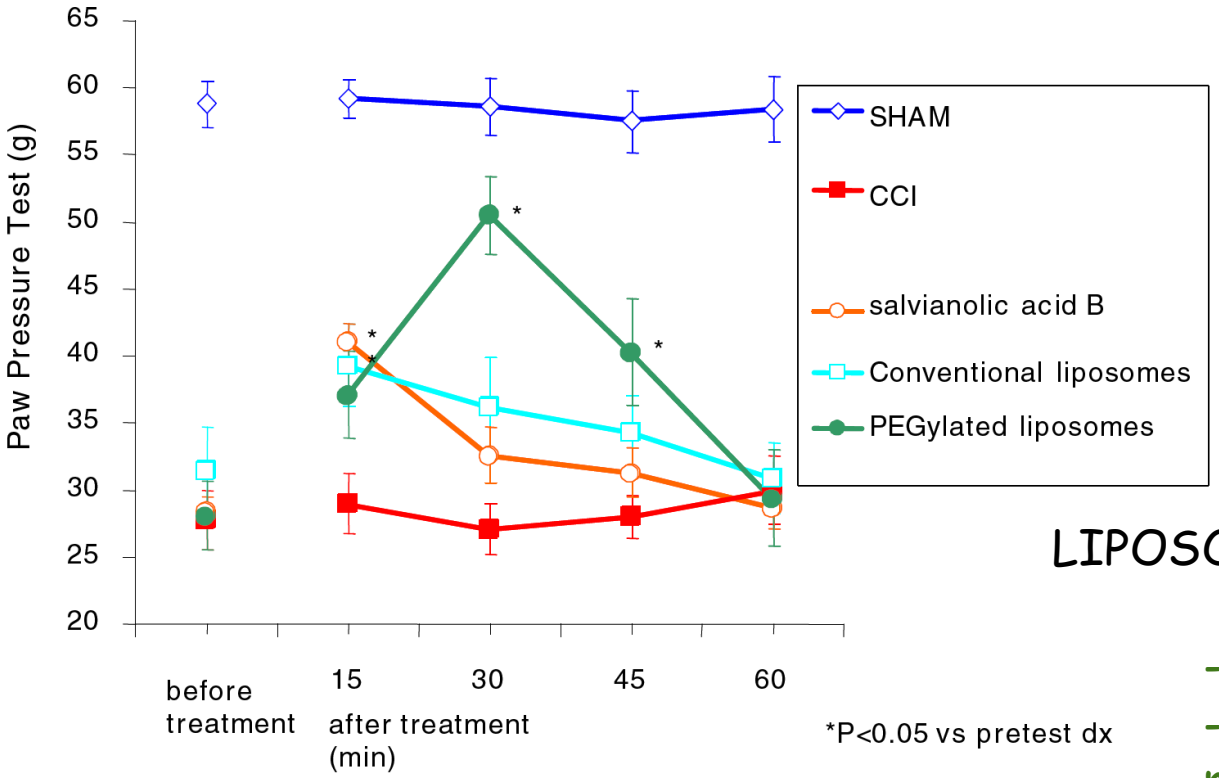
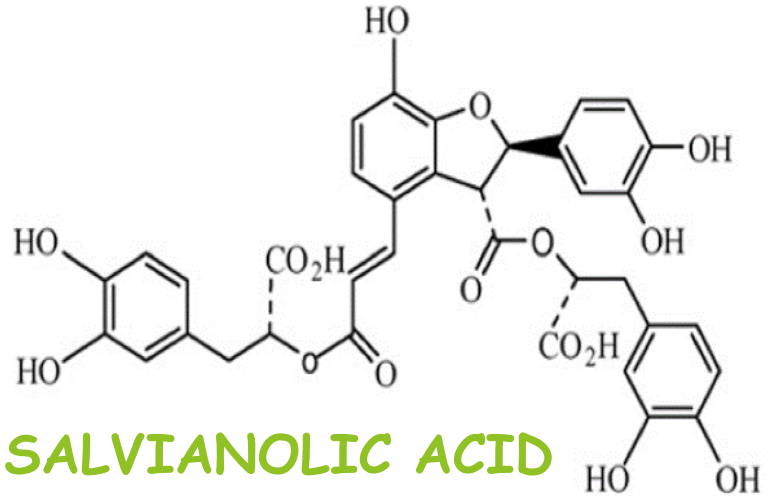






SalB-loaded liposomes intraperitoneal administration in a chronic constriction injury of the sciatic nerve (CCI) in the rat paw-pressure test.



SALVIANOLIC ACID

LIPOSOMES

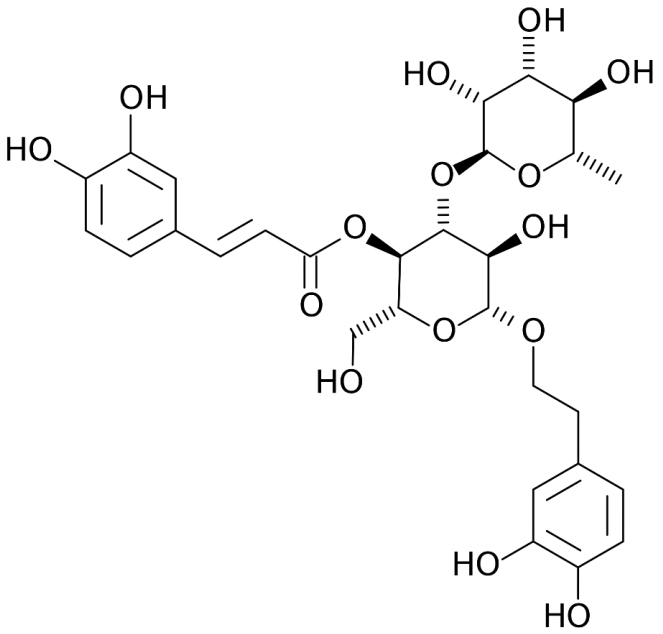
-protect from hydrolisis
-improve bioavailability giving a more effective antihyperalgesic activity

Liposomal Formulation to Increase Stability and Prolong Antineuropathic Activity of Verbascoside

Authors Benedetta Isacchi[1]*, Maria Camilla Bergonzi[1]*, Romina Iacopi[1], Carla Ghelardini[2], Nicoletta Galeotti[2], Anna Rita Bilia[1]

Affiliations
[1] Department of Chemistry, University of Florence, Sesto Fiorentino (FI), Italy
[2] Department of Neuroscience, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy

VERBASCOSIDE



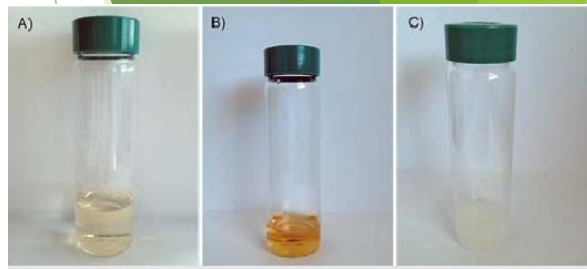


Fig. 3 Macroscopic degradation of verbascoside. Fresh verbascoside solution (**A**); degraded verbascoside solution (**B**); verbascoside-loaded liposomes (**C**). (Color figure available online only.)

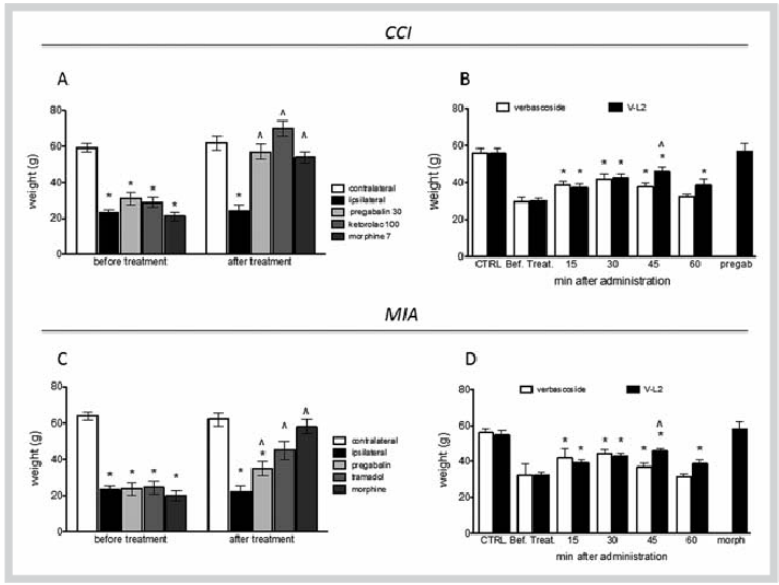


Fig. 4 Positive controls used as reference compounds in a chronic constriction injury of the sciatic nerve (CCI; **A**) and in an intra-articular injection of sodium monoiodoacetate (MIA; **C**) in the rat paw pressure test. Values were recorded 30 min after the beginning of the test. * $p < 0.05$ vs. contralateral side; ^ $p < 0.05$ vs. ipsilateral side. Effect of verbascoside and of verbascoside-loaded liposomes V-L2 in a chronic constriction injury of the sciatic nerve (**B**) and in an intra-articular injection of sodium monoiodoacetate (**D**) on rat pain models evaluated in the paw pressure test. Preagabalin and morphine were used as control reference drugs. * $p < 0.05$ vs. before treatment (Bef. Treat.) values; ^ $p < 0.05$ vs. corresponding verbascoside-treated group. Data represent the mean + SEM of n = 10 animals per group.

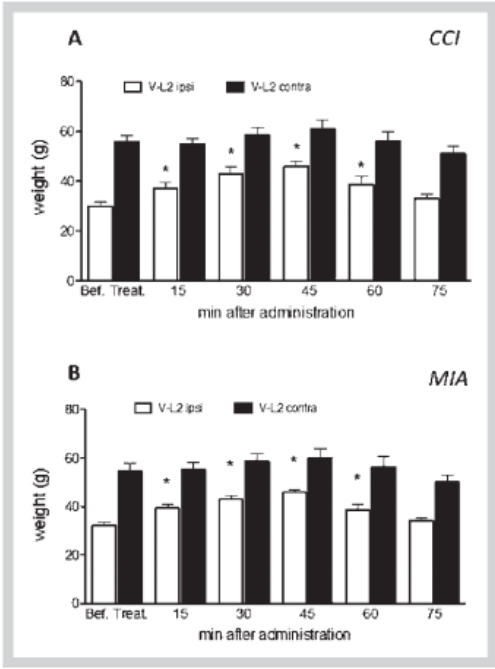
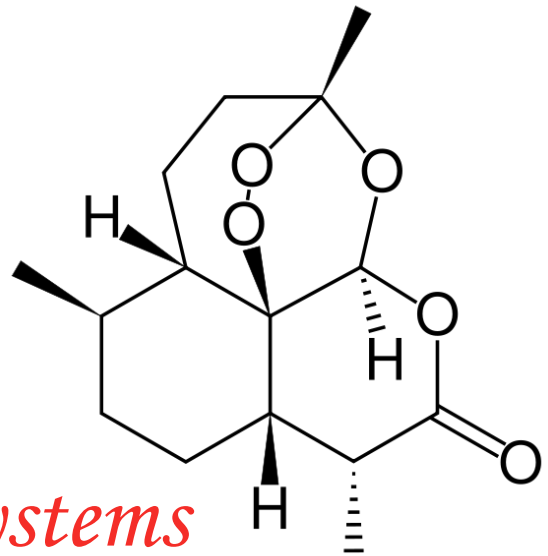


Fig. 5 Time-course curve of V-L2 in the CCI (**A**) and MIA (**B**) models. *$p < 0.05$ vs. ipsilateral before treatment (Bef. Treat.). Data represent the mean + SEM of n = 10 animals per group.

Limiting factors

- low solubility
- quickly metabolized in vivo
- initial burst effect and high peak concentration
- not stable
- short-duration effect

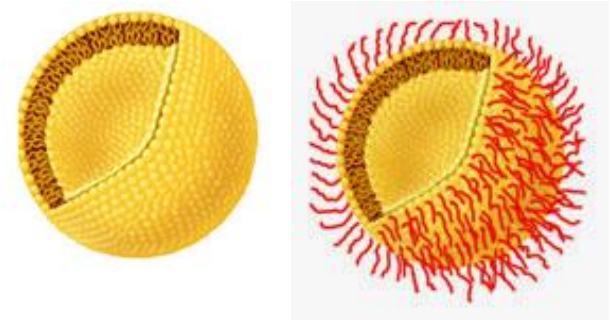
ARTEMISININ



Drug delivery systems

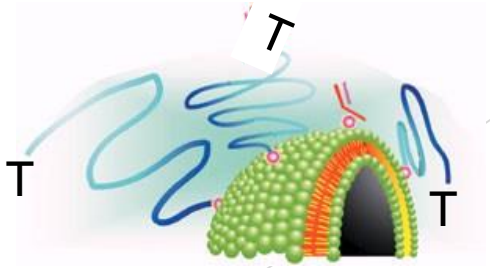
Passive targeting:

conventional and stealth liposomes

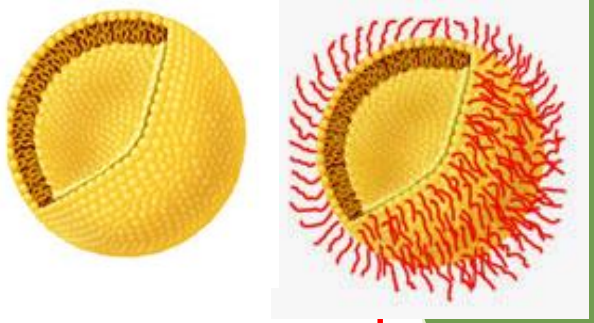


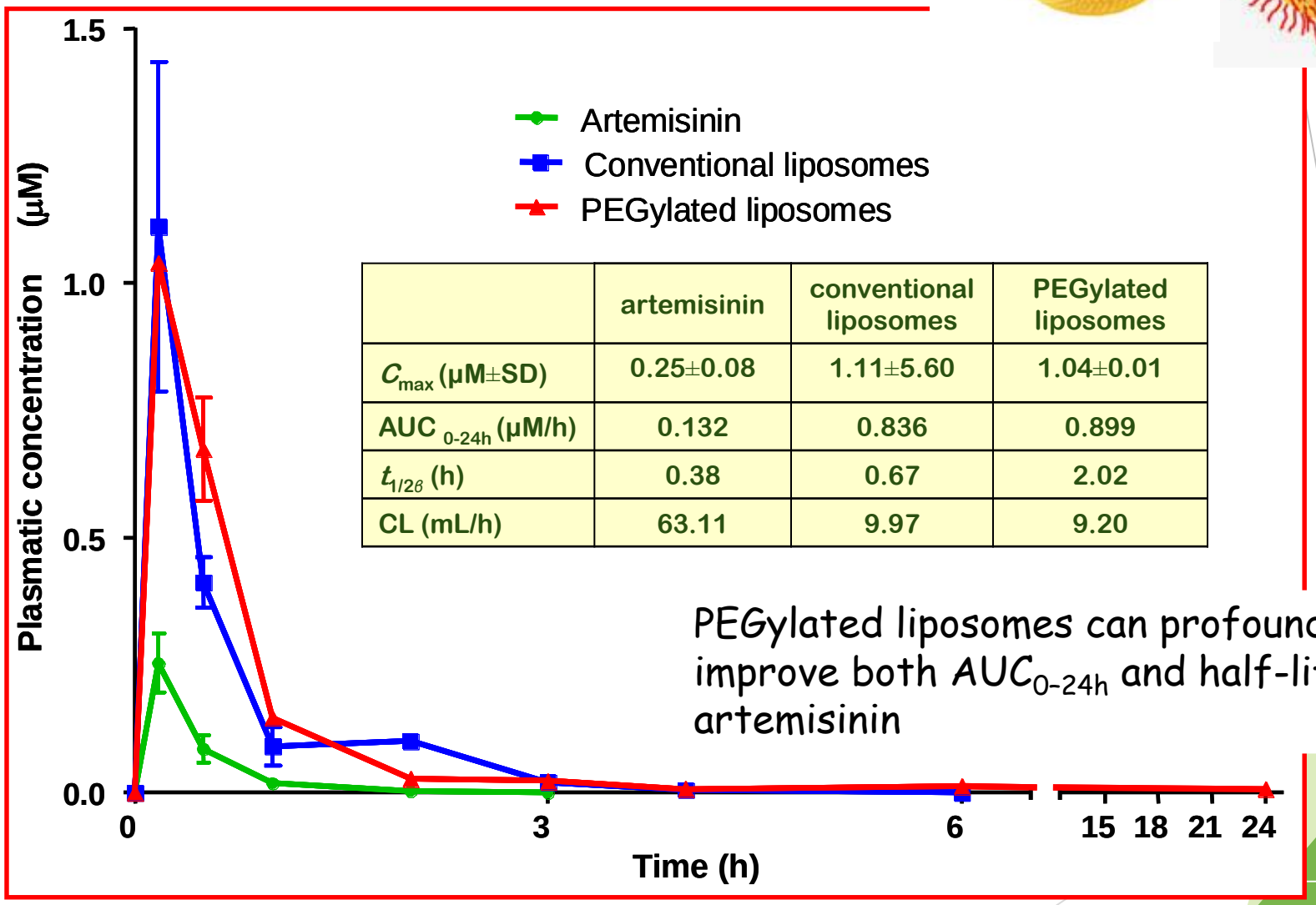
Active targeting:

Transferrin conjugated liposomes



Pharmacokinetic profiles in mice



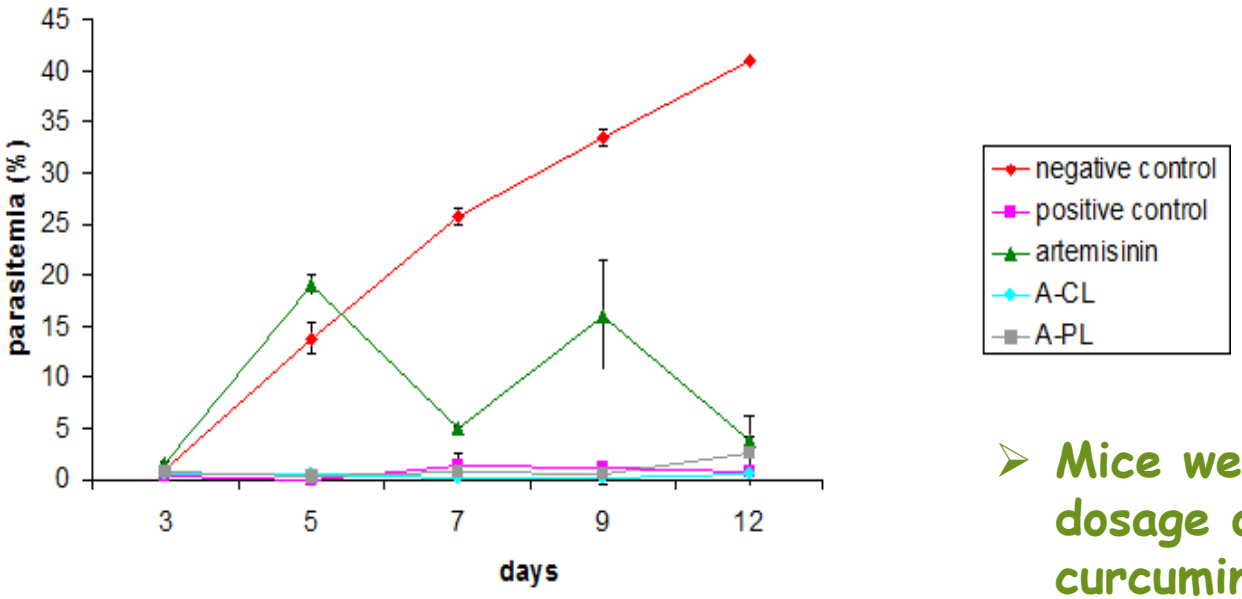


	artemisinin	conventional liposomes	PEGylated liposomes
C_{max} (μM±SD)	0.25±0.08	1.11±5.60	1.04±0.01
AUC $_{0\text{-}24h}$ (μM/h)	0.132	0.836	0.899
$t_{1/2\beta}$ (h)	0.38	0.67	2.02
CL (mL/h)	63.11	9.97	9.20

PEGylated liposomes can profoundly improve both $AUC_{0\text{-}24h}$ and half-life of artemisinin

Isacchi et al., J. Lipos. Res. (2011) 20: 1–8.

Antimalarial activity in mice



European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 528–534

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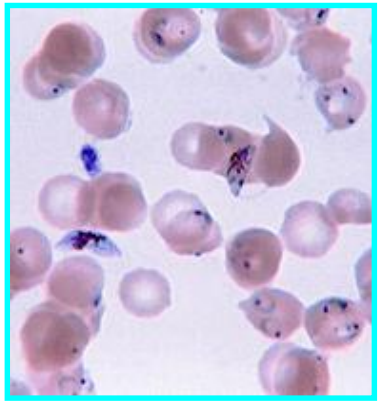
Research paper

Artemisinin and artemisinin plus curcumin liposomal formulations: Enhanced antimalarial efficacy against *Plasmodium berghei*-infected mice

Benedetta Isacchi [a,*], Maria Camilla Bergonzi [a], Margherita Grazioso [a], Chiara Righeschi [a], Alessia Pietretti [b], Carlo Severini [b], Anna Rita Bilia [a]

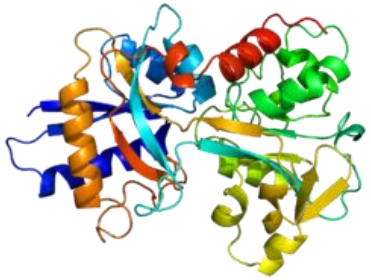
[a] Department of Pharmaceutical Sciences, University of Florence, Sesto Fiorentino, Italy
[b] Department of Infectious, Parasitic and Immunomediated Disease, Vector-Borne and International Health Section, Istituto Superiore di Sanità, Rome, Italy



P. berghei

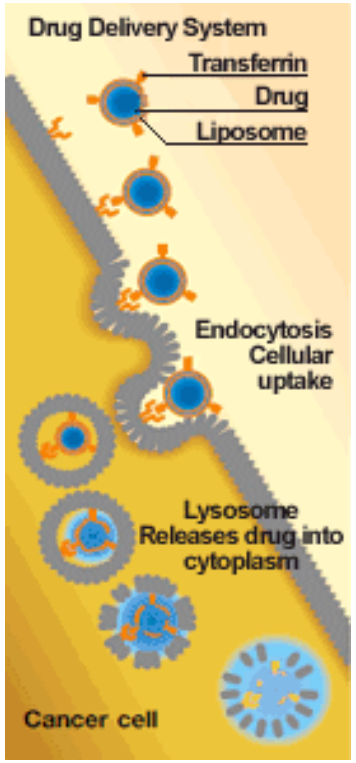
NK 65 strain

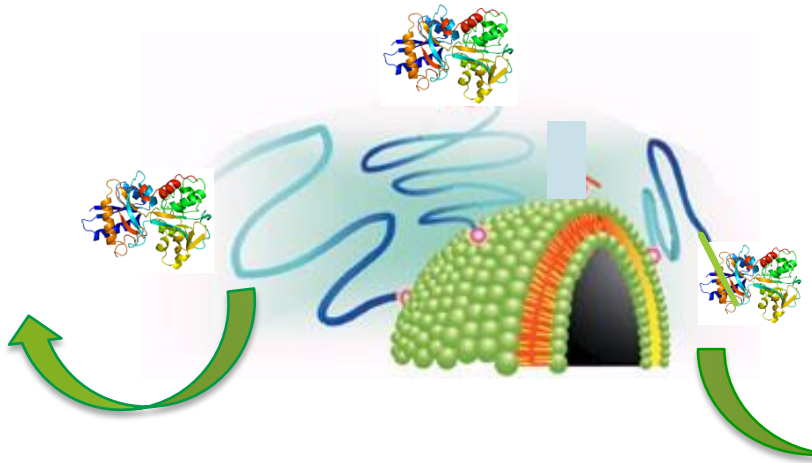
- Mice were treated with artemisinin at the dosage of 50 mg/kg/day alone or plus curcumin as partner drug, administered at the dosage of 100 mg/kg/day.
- Only treatments with artemisinin-loaded conventional or PEGylated liposomes appeared to have an immediate antimalarial effect.
- Artemisinin-loaded liposomes are reasonable delivery strategies to prolong circulating time in blood due to the passive targeting
- All the liposomal treatments extended the period of survival of the mice until 30 days post-inoculation.



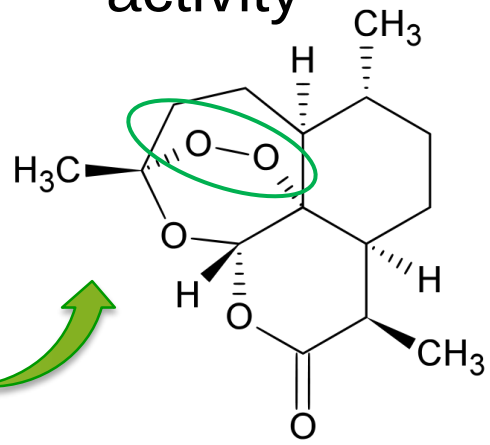
TRANSFERRIN CONJUGATED LIPOSOMES FOR ARTEMISININ DELIVERY

Enhanced selectivity





Enhanced activity



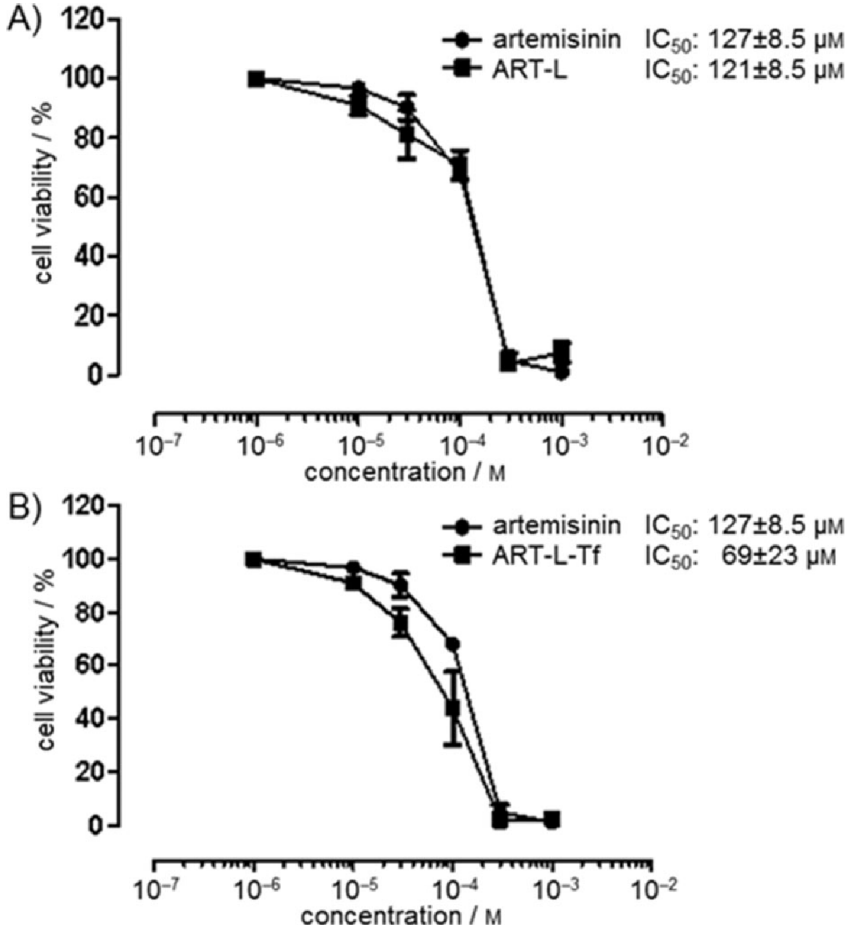
- Transferrin is a medium size protein with two domains where iron binds to a cavity in each domain. The level of transferrin receptor expression varies depending on cell types.

- Most cancer cells express a high concentration of transferrin receptors on cell surface and have a high amount of Fe(III) ion uptake into cells. Because cancer cells have higher iron influx rates compared to the corresponding normal cells, cancer cells are more susceptible to the cytotoxic effect of artemisinin.

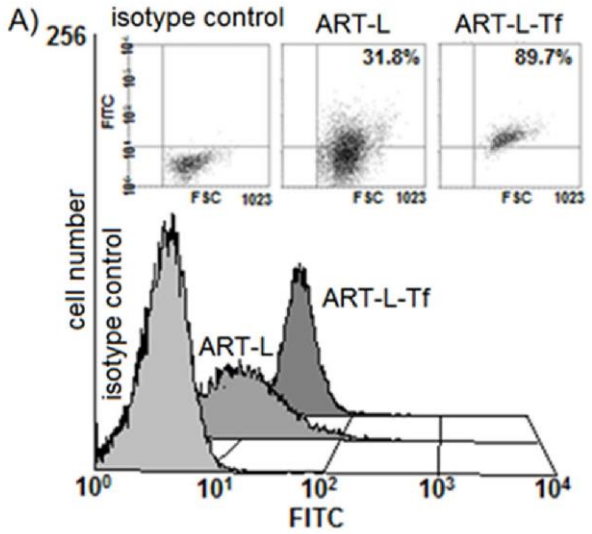
ChemPubSoc Europe
DOI: 10.1002/cmdc.201500586
CHEMMEDCHEM Full Papers

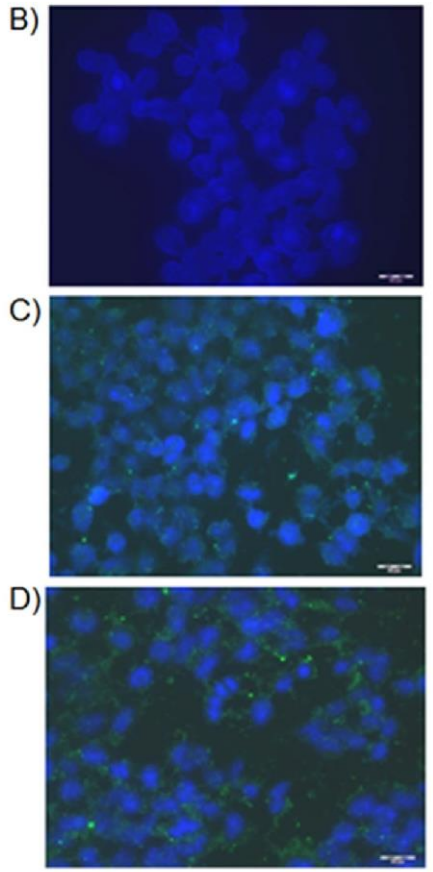
Enhanced Efficacy of Artemisinin Loaded in Transferrin-Conjugated Liposomes versus Stealth Liposomes against HCT-8 Colon Cancer Cells

Isabella Leto,[a] Marcella Coronnello,[b] Chiara Righeschi,[a] Maria Camilla Bergonzi,[a] Enrico Mini,[b] and Anna Rita Bilia*[a]



In vitro cytotoxicity of free ART, ART-L, and ART-L-Tf on HCT-8 colon cancer cells as a function of drug concentration. Data are expressed as a percentage of cell viability as evaluated by sulforhodamine B test.





Cellular uptake of liposomes detected by flow cytometry. A) Histograms related to HCT-8 cells treated with isotype control, ART-L, and ART-LTf. Insets show biparametric analysis of the cells (FSC versus NBD-PE-stained cells) and the percentage of stained cells. Panels B) HCT-8 isotype control, C) (ART-L), and D) (ART-L-Tf) show images obtained by fluorescence microscopy

POLYMERIC NANOPARTICLES

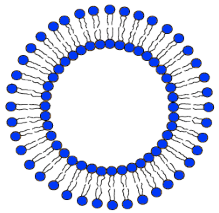
ALBUMIN (HSA) NANOSPHERE

POLY ETHYLCYANOACRYLATE (PECA) NANOSPHERE

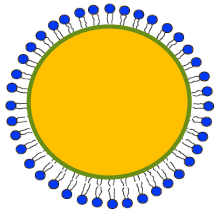
Polymer chain

LIPID-BASED NANOPARTICLES

VESICLE

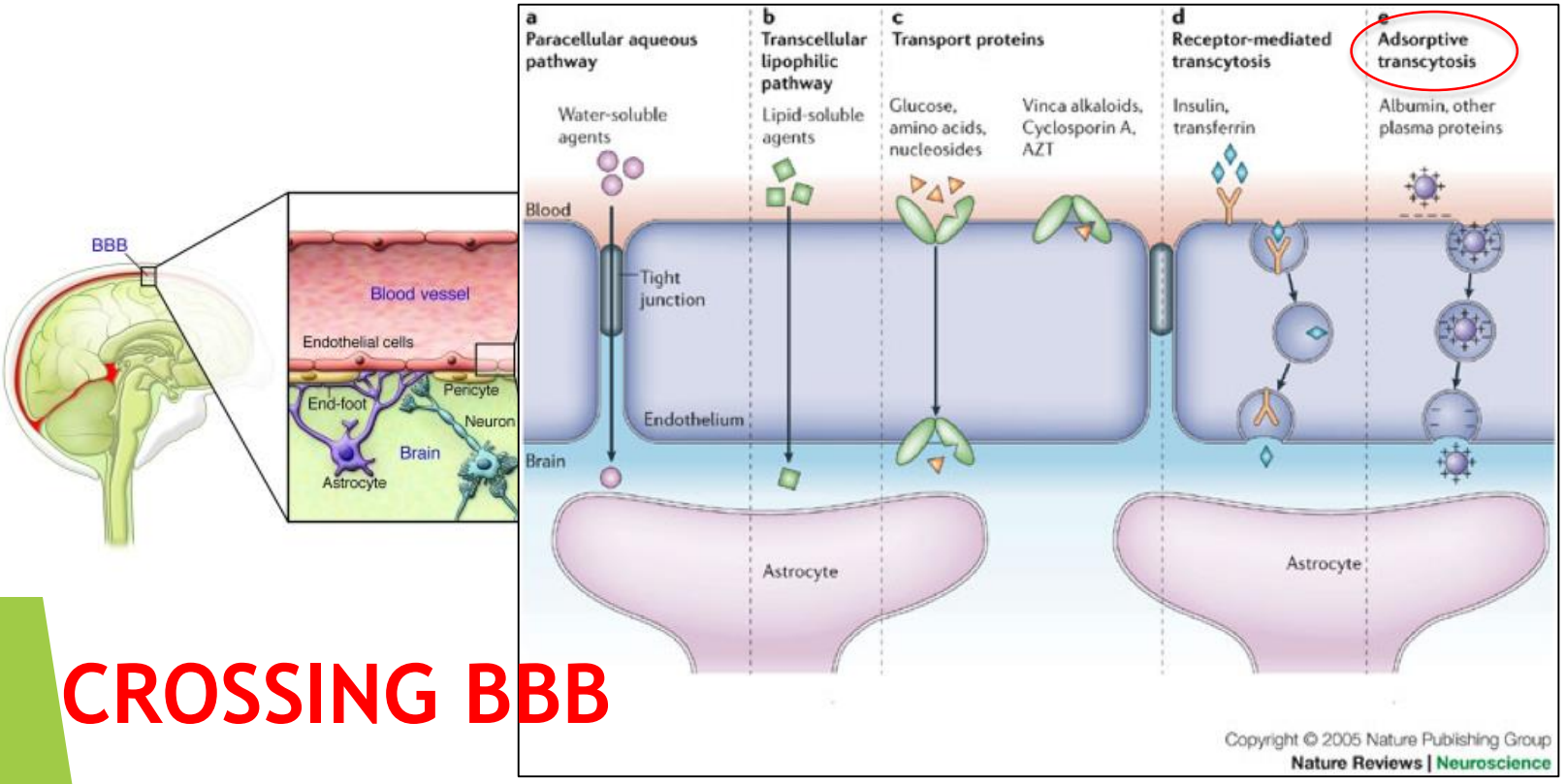


SOLID LIPID NANOPARTICLE



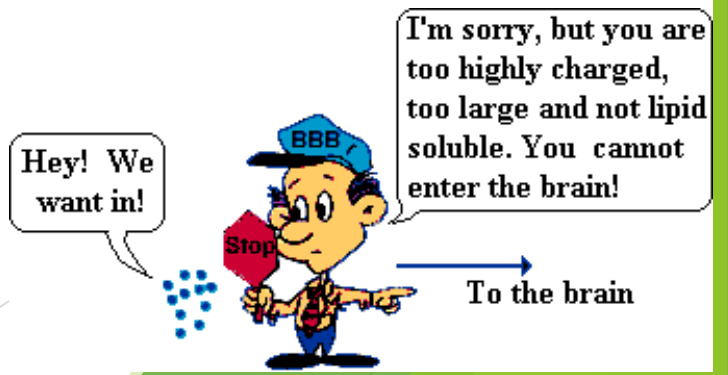
DEVELOPED NANOCARRIERS

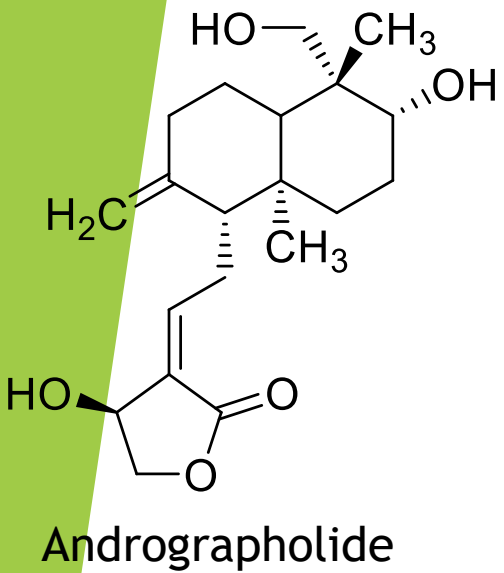
HSA NPs



CROSSING BBB

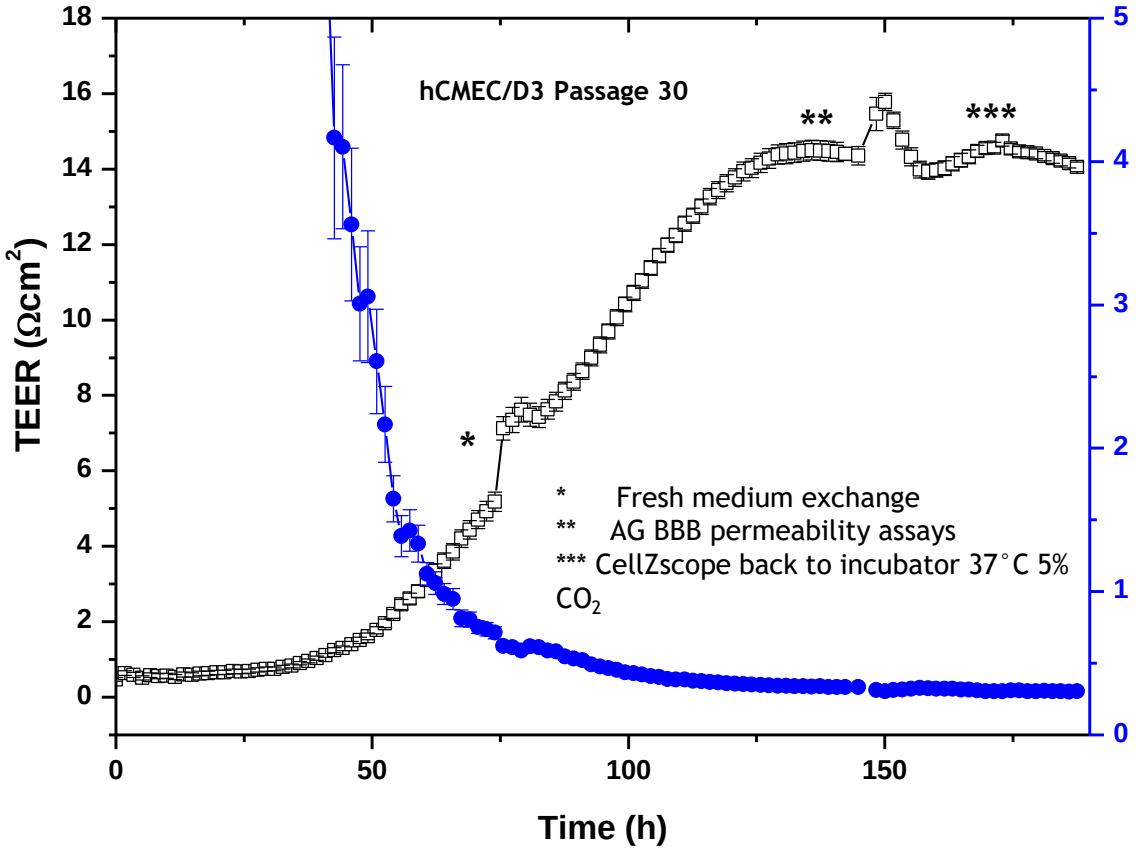
- **Bergonzi et al.** Albumin Nanoparticles for Brain Delivery: A Comparison of Chemical versus Thermal Methods and in vivo Behaviour. ChemMedChem. 2016 Aug 19;11(16):1840-9. doi: 10.1002/cmdc.201600080.
- **Grossi C,** Guccione C, Isacchi B, Bergonzi MC, Luccarini I, Casamenti F, **Bilia AR.** Development of Blood-Brain Barrier Permeable Nanoparticles as Potential Carriers for Salvianolic Acid B to CNS. Planta Med. 2017 Mar;83(5):382-391. doi: 10.1055/s-0042-101945. Epub 2016 Mar 22.
- **Guccione et al.** Andrographolide-loaded nanoparticles for brain delivery: Formulation, characterisation and in vitro permeability using hCMEC/D3 cell line. Eur J Pharm Biopharm. 2017 Oct;119:253-263.
- **Piazzini et al.** Stealth and Cationic Nanoliposomes as Drug Delivery Systems to Increase Andrographolide BBB Permeability. Pharmaceutics 2018, 10, 128;
- **Graverini et al.** Solid lipid nanoparticles for delivery of andrographolide across the blood-brain barrier: in vitro and in vivo evaluation. Colloids Surf B Biointerfaces. 2018 Jan 1;161:302-313.
- **Bilia et al.** Successful Brain Delivery of Andrographolide Loaded in Human Albumin Nanoparticles to TgCRND8 Mice, an Alzheimer's Disease Mouse Model. Front Pharmacol. 2019 Aug 22;10:910. doi: 10.3389/fphar.2019.00910.





Andrographolide

BBB drug permeability assays with free AG at 5 µM and Na-F at 10µg/mL



European Journal of Pharmaceutics and Biopharmaceutics 119 (2017) 253–263

Contents lists available at ScienceDirect

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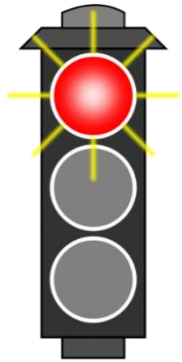
Research paper

Andrographolide-loaded nanoparticles for brain delivery: Formulation, characterisation and *in vitro* permeability using hCMEC/D3 cell line

Clizia Guccione [a,*,1], Mouhssin Oufir [b,1], Vieri Piazzini [a], Daniela Elisabeth Eigenmann [b], Evelyn Andrea Jähne [b], Volha Zabela [b], Maria Teresa Faleschini [b], Maria Camilla Bergonzi [a], Martin Smiesko [c], Matthias Hamburger [b], Anna Rita Bilia [a]

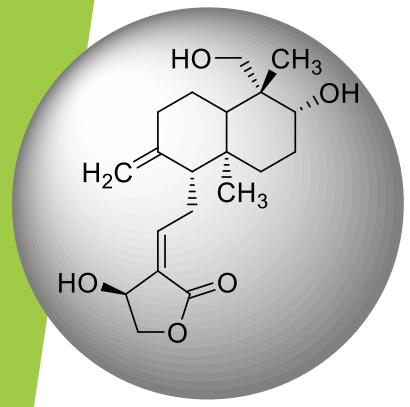


Immortalized human endothelial cell line (hCMEC/D3) BBB model (n=3)

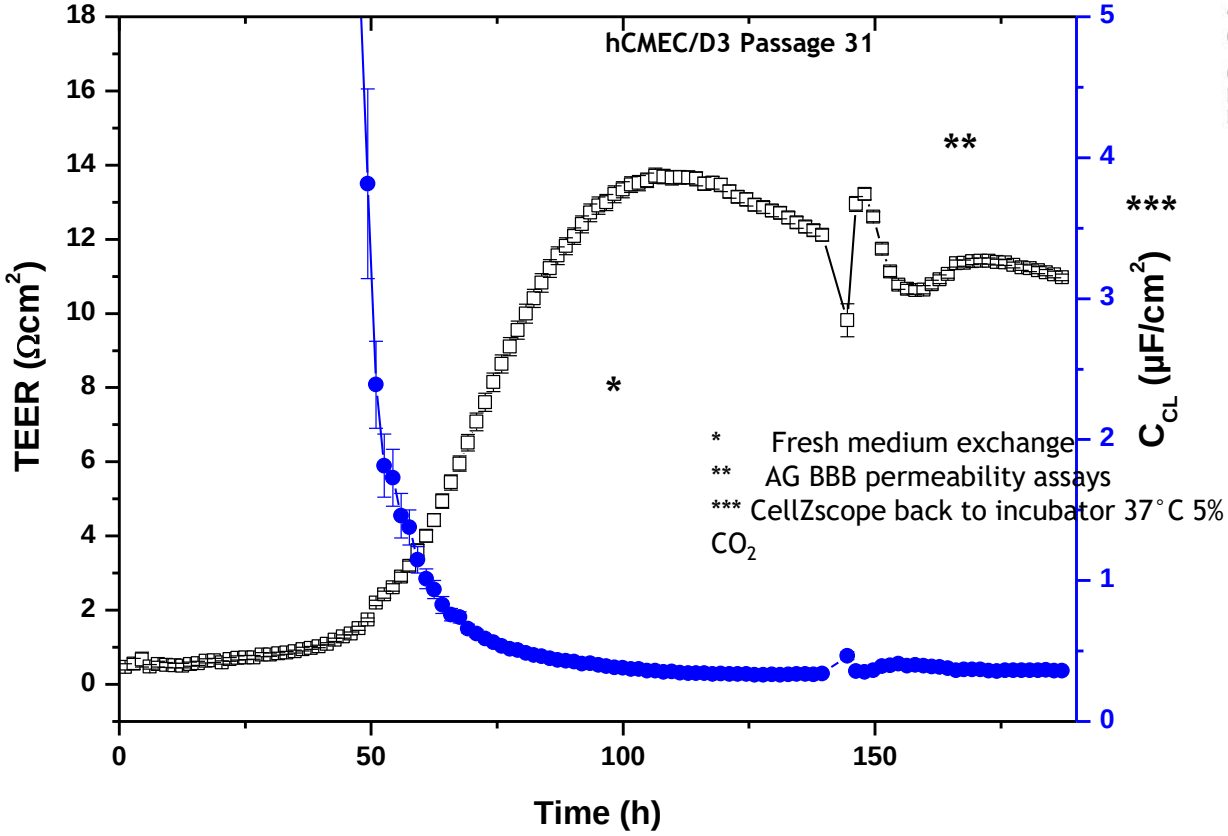
Transport direction	Δt (min)	P_{app} AG ± S.E.M ($x10^{-6}$ cm/s)	P_{app} Na-F ± S.E.M ($x10^{-6}$ cm/s)
A → B	60	9.51	7.85

AG DOES NOT CROSS THE BBB

BBB drug permeability assays with HSA nanoparticles loaded with AG at 50 µM and and Na-F at 10µg/mL



HSA NP



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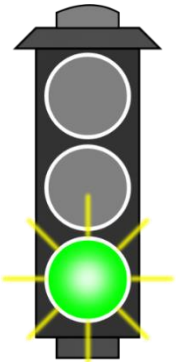
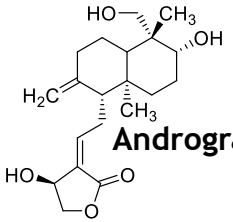


Immortalized human endothelial cell line (hCMEC/D3) BBB model (n=3)

Transport direction	Δt (min)	P_{app} AG ± S.E.M ($x10^{-6}$ cm/s)	P_{app} Na-F ± S.E.M ($x10^{-6}$ cm/s)
A → B	60	18.7	9.34

HSA NPs IMPROVE THE PERMEABILITY OF AG ACROSS THE BBB

ALBUMIN NPs



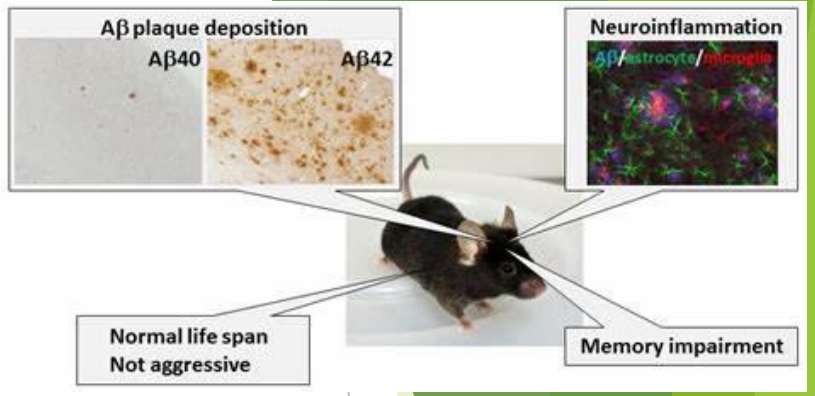
Andrographolide

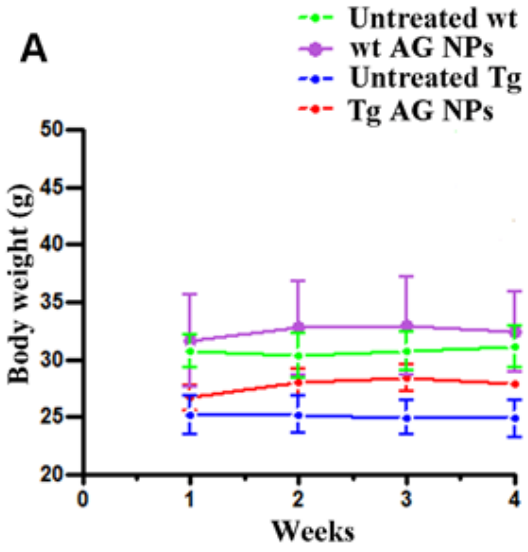
FORMULATION

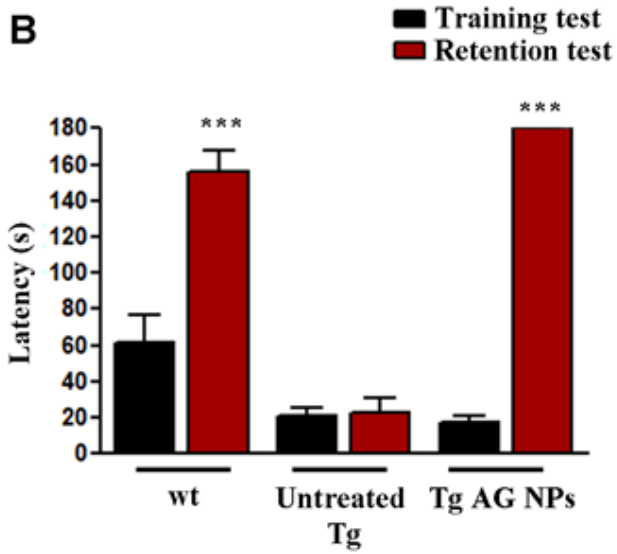
In vivo studies TgCRND8 mice, an Alzheimer's disease mouse model

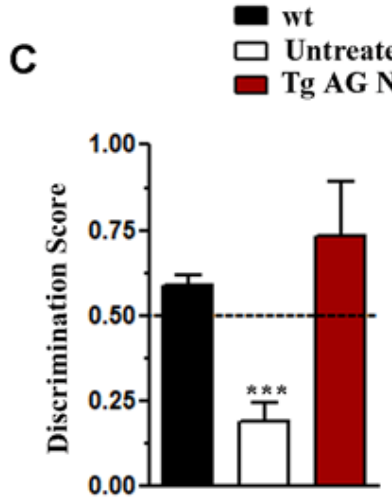
<u>ABILITY TO CROSS BBB:</u>
Intraperitonel and intravenous administration

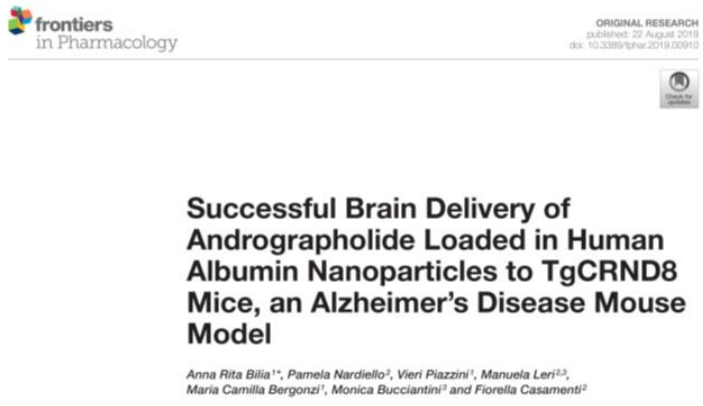
<u>EFFICACY PROFILES:</u>
Behavioural studies in mice










frontiers in Pharmacology

ORIGINAL RESEARCH

Successful Brain Delivery of Andrographolide Loaded in Human Albumin Nanoparticles to TgCRND8 Mice, an Alzheimer's Disease Mouse Model

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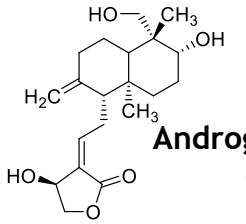
NPs were well tolerated and no evident side effects were revealed, as shown by the body weight trend graph (A) and no animals died. **Potential effects of NPs on cognitive functions and locomotor-exploratory abilities were tested in the step down (B) and object recognition test (ORT, C) behavioural tests.** In the step down inhibitory avoidance test (B) latencies observed for untreated Tg mice, in the step down RT, were significantly reduced compared to ones observed for wt mice (***P<0.0001). AG NPs treatment to Tg mice significantly improved their performance (***P<0.0001, vs untreated Tg mice) to levels comparable to those displayed by wt mice. In the ORT (C), treated and untreated animals showed no deficiencies in exploratory activity, directional movement towards the objects and locomotor activity and no cognitive impairments (discrimination score) were detected in AG NPs treated Tg mice (***P<0.0001, versus untreated Tg mice)

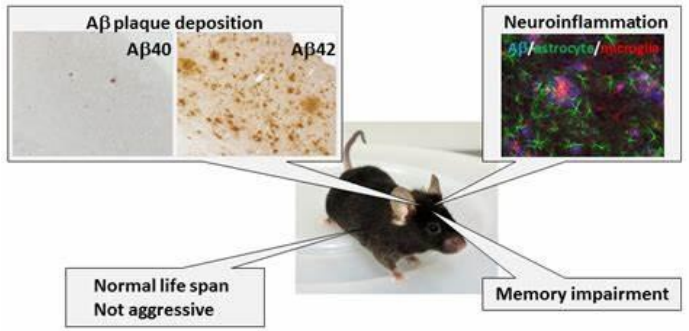
ALBUMIN NPs



Andrographolide (AG)

FORMULATION

In vivo studies TgCRND8 mice, an Alzheimer's disease mouse model

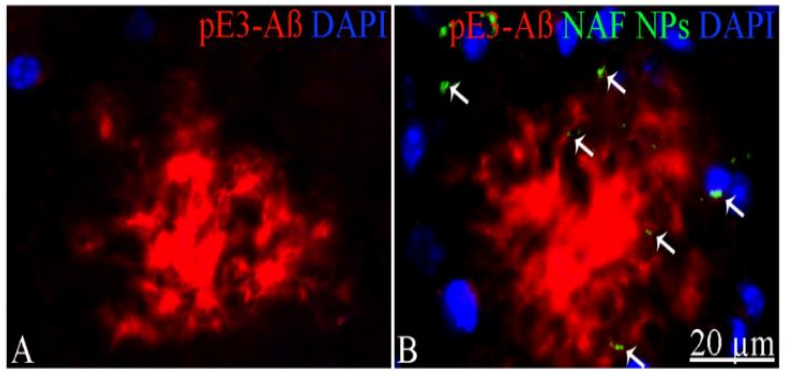


ABILITY TO CROSS BBB:

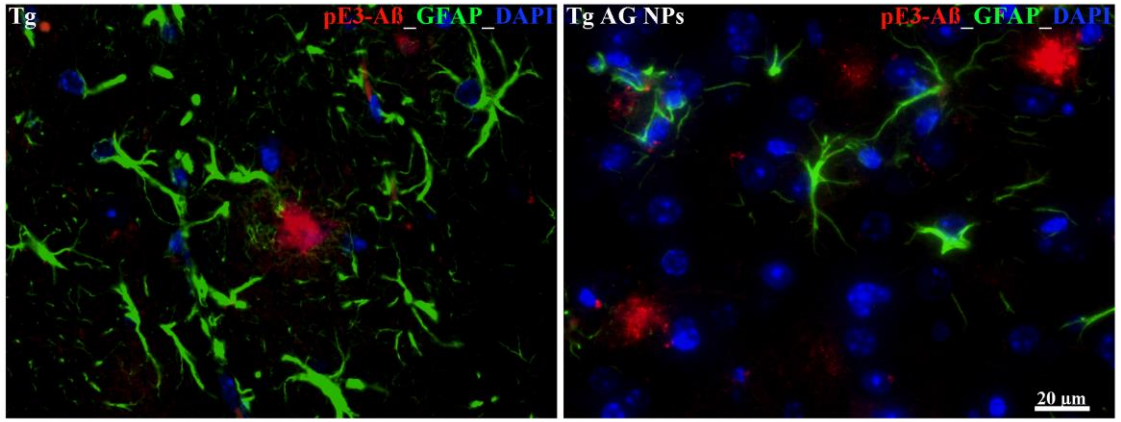
Intraperitonel and intravenous administration

EFFICACY PROFILES:

Behavioural studies in mice



The penetration into the brain of NAF NPs (green), 200 µL administered acutely i.v., was investigated. Three hours after the administration, the formulation was detected in the brain parenchyma (arrows) of Tg mice. Interestingly, immunofluorescent analyses with the N3pE antibody, that recognizes pE3-Aß plaque, NAF-loaded NPs (arrows) were detected both in the pE3-Aß plaque (red) surroundings and inside the pE3-Aß plaque, this is indicative of the **ability of these nanoformulations to cross the BBB and to penetrate in undamaged and damaged brain tissue.**



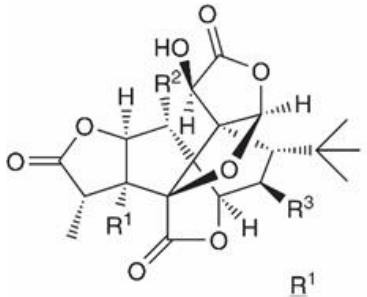
Immunohistochemical analysis of GFAP positive astrocytes in the hippocampus of Tg mice. Left panel) untreated (Tg); right panel) treated (AG NPs). GFAP staining (green) revealed less reactive astrocytes, characterized by enlarged cell body, in the pE3-Aß plaque (red) surroundings of treated (AG NPs) mice, compared to untreated (Tg) mice. DAPI is in blue. **AG NPs induced amelioration of cognitive functions was associated with a reduced astrocytes reaction, revealing fewer reactive astrocytes with enlarged cell body in the pE3-Aß plaque (red) surroundings and brain parenchyma in the hippocampus of AG NPs administered Tg mice, compared to untreated Tg mice.** Strong evidence for the AG NPs efficacy on cognitive functions and further support the anti-inflammatory activity of AG

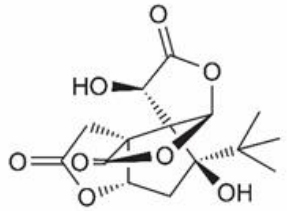
Bilia et al., Successful brain delivery of andrographolide loaded in human albumin nanoparticles to TgCRND8 mice, an Alzheimer's disease mouse model. Frontiers in Pharmacology, special issue "Novel Targets and the Application of Targeting Techniques in the Treatment of Cerebrovascular Diseases"

Ginkgo biloba L.

Ginkgolides and bilobalide: 6-8%
Flavonoids 25-27%
100-(8+27)=65% ?

Diterpenes

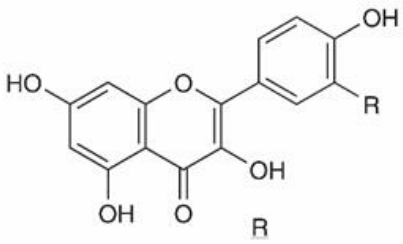




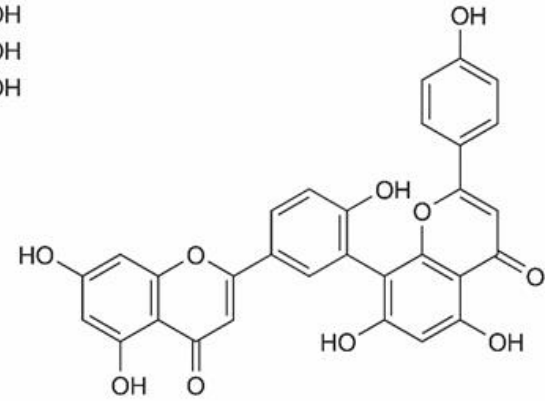
bilobalide

		R^1	R^2	R^3
ginkgolide	A	OH	H	H
ginkgolide	B	OH	OH	H
ginkgolide	C	OH	OH	OH
ginkgolide	J	OH	H	OH
ginkgolide	M	H	OH	OH

Flavonoids

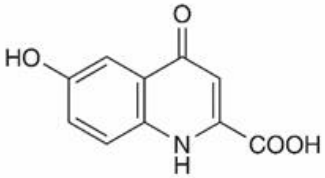


	R
kaempferol	H
quercetin	OH

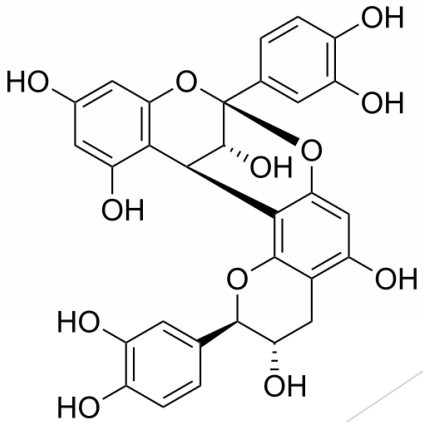


amentoflavone

Amino acids



6-hydroxykynurenic acid

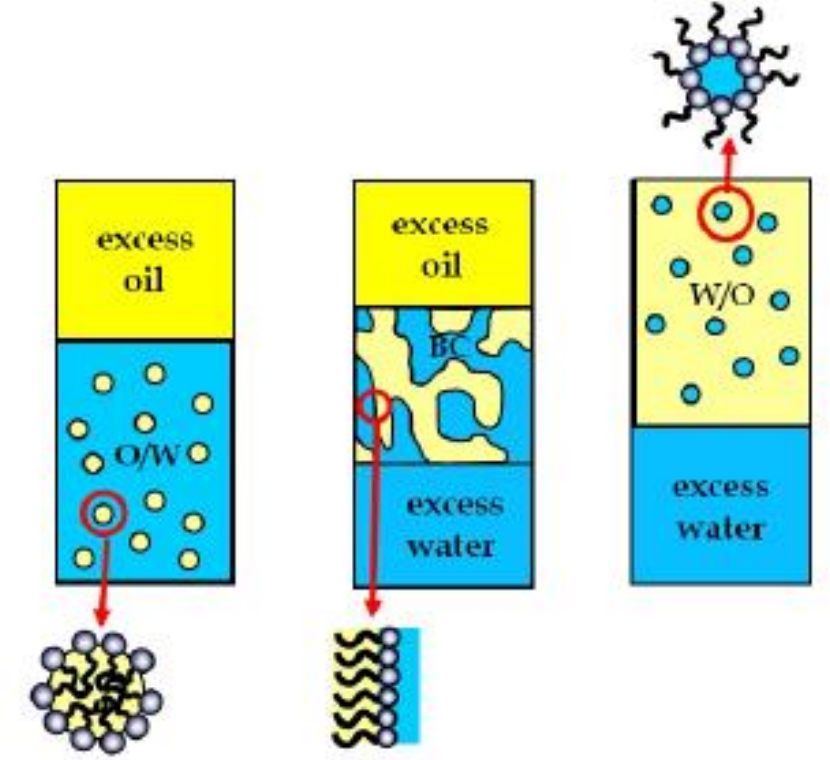


procyanidin

EXTRACTS

NANOEMULSIONS/MICROEMULSIONS

- Microemulsion can form spontaneously and are thermodynamically stable, nanoemulsions need energy for emulsification but are kinetically stable
- highly dispersed, stable, transparent formulations and easy to prepare
- their nanosized dimensions allow a better absorption by the cells
- ability to solve the problems of solubility and stability of many extracts
- to formulate the extract as both aqueous solutions and as non-aqueous concentrates, diluted with water immediately before administration, or administered as such.



EMULSION NANOEMULSION/ MICROEMULSION

SELF-MICROEMULSING DRUG DELIVERY SYSTEMS (SMEDDs)

MICROEMULSIONS

System	Amount of extract (%)
Nano/Microemulsion	0.5-20
Nanoparticle	0.5-2.0
Vesicle and nanocochleate	0.1-2.0

Amount of extract (dry weight) usually associated to each nanotechnology-based system.

DER 8.0-14.3:1,containing not less than 70.0 percent and not more than 95.0 percent of fatty acids and not less than 0.2 percent and not more than 0.5 percent of sterols, calculated on an anhydrous basis

pineapple (*Ananas comousus* L.) stem aqueous extract

nettle (*Urtica urens* L./*Urtica dioica* L.) root dried extract

(70% V/V EtOH, DER 12-16:1, containing 0.82% β-sitosterol)

Not homogeneous formulation can affect dissolution, solubility, and allow for an adequate and reproducible absorption from the gastrointestinal tract following oral administration

COMMERCIAL BLEND OF SAW PALMETTO; NETTLE AND PINEAPPLE IN THE FORM OF SOFT GEL CAPSULES

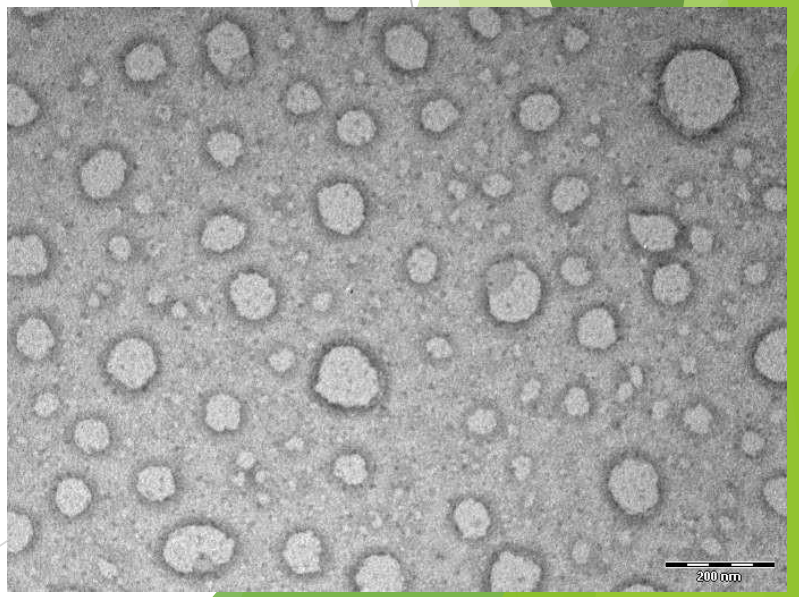
Original Papers

Thieme

Lipid Nanocarriers for Oral Delivery of *Serenoa repens* CO_2 Extract: A Study of Microemulsion and Self-Microemulsifying Drug Delivery Systems

Authors
Clizia Guccione, Maria Camilla Bergonzi, Khaled M. Awada, Vieri Piazzini, Anna Rita Bilia

STRATEGY: microemulsions and self-emulsifying drug delivery systems (SEDDS)

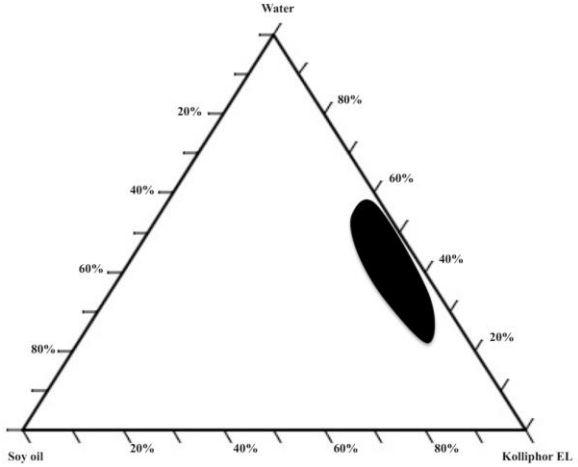


	Solubility of saw palmetto extract (%)
Almond oil	52.78
Soybean oil	64.44
Vitamin E	56.11
Sunflower oil	56.33
Oleic acid	56.11
Cannabis sativa seed oil	61.67
Borrago officinalis seed oil	68.33
Labrafil	52.22
Capryol 90	65.56
Triacetin	11.11
Argania spinosa kerne oil	57.22
Tween 80	67.22
Tween 20	60.56
Transcutol HP	48.33
Kolliphor EL	67.78
Diclorometane:MeOH 1:1	100
Water	-

Solubility of the SR fluid extract in various oils, surfactants and DCM:MeOH

Nanocarrier	Size (nm)	Polydispersity	ζ- potential (mV)
M1	14.6 ±0.04	0.2±0.02	-46.5±0.21
M2	19.5 ±0.01	0.2±0.01	-17.9±0.11
CBM1	250.4±0.22	0.3±0.10	-18.2±0.31
CBM2	200.9±0.01	0.3±0.06	-46.7±0.02
CBS1	399.7±0.34	0.6±0.09	-19.2±0.45
CBS2	239.0±0.11	0.3±0.03	-27.1±0.28

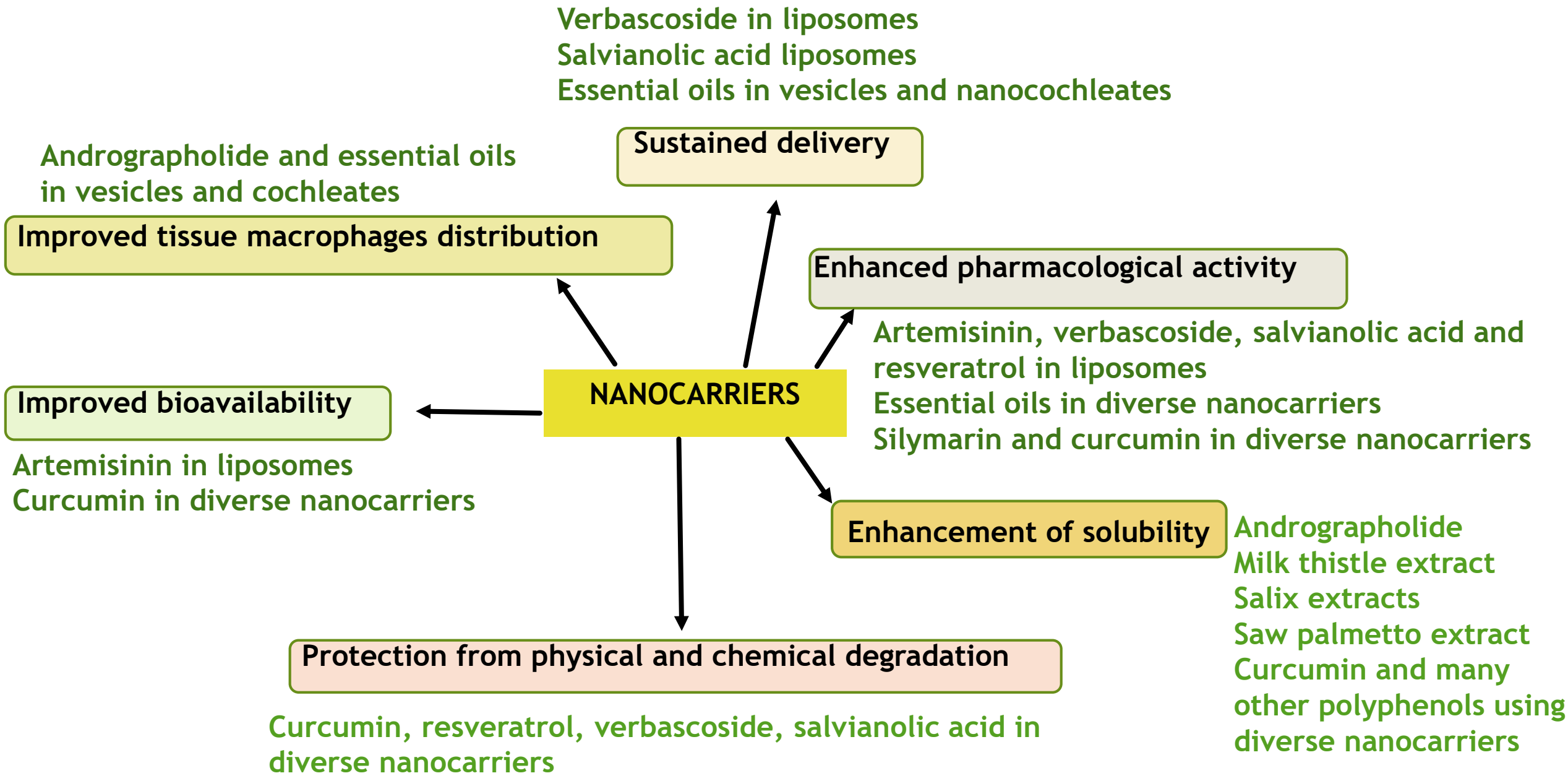
DLS characterization of MEs (M1, M2, CBM1 and CBM2) and SMEDDSs (CBS1 and CBS2) in terms of size (nm), polydispersity and ζ- potential (mV). (Mean ± S.D.; *n*=3)



Pseudoternary phase diagram of CBM2. The black area represents microemulsion region.

PAMPA test.

Sample	Time	% permeation	% of recovery
CBM2	2h	nd	-
	4h	2%	99%
	6h	17%	99%
CBS2	2h	2%	87%
	4h	4%	83%
	6h	7%	78%
commercial blend	2h	nd	-
	4h	nd	-
	6h	1%	86.5%
saw palmetto extract	2h	nd	-
	4h	nd	-
	6h	3%	98%


Verbascoside in liposomes
Salvianolic acid liposomes
Essential oils in vesicles and nanocochleates
Sustained delivery
Andrographolide and essential oils in vesicles and cochleates
Improved tissue macrophages distribution
Enhanced pharmacological activity
Artemisinin, verbascoside, salvianolic acid and resveratrol in liposomes
Essential oils in diverse nanocarriers
Silymarin and curcumin in diverse nanocarriers
Improved bioavailability
Artemisinin in liposomes
Curcumin in diverse nanocarriers
NANOCARRIERS
Enhancement of solubility
Andrographolide
Milk thistle extract
Salix extracts
Saw palmetto extract
Curcumin and many other polyphenols using diverse nanocarriers
Protection from physical and chemical degradation
Curcumin, resveratrol, verbascoside, salvianolic acid in diverse nanocarriers

Message to take home

- **NATURAL PRODUCTS** are pleiotropic molecules, generally able to influence numerous biochemical and molecular cascades, representing a realistic approach to many diseases, especially those with emerging resistance to monofunctional agents, and multifactorial and complex diseases, especially cancer and diabetes.
- **NANOCARRIERS** could optimise their solubility, stability and bioefficacy
- **NATURAL PRODUCTS** can represent both the actives and the constituents of the structure/architecture of the carrier
- Isolated constituents are easy to formulate when compared with extract, but difficulties are depending on the extracts (hydroethanolic extracts, carbon dioxide extracts, essential oils)
- Extracts should be defined according the chemical profile, DER, this is the most important step

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YoungInnovation: the state of research communicated by young researchers – Nanotechnologies meet natural products

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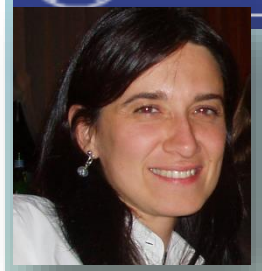
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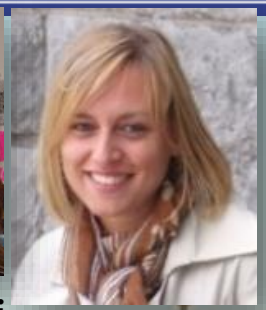
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Thank you!