Olive Lifesciences

Improving Lives…….

BioTurmin™

Technical Document V1.2

SINCE 2007

Turmeric Extract- An Introduction

Botanical classification

- Botanical name - *Curcuma longa*
- Family: Zingiberaceae
- Genus: Curcuma
- Species: Longa
- Plant part used : Rhizomes
- CAS No. 84775-52-0
- HS Code: 13021919

Applications

- Nutraceutical and Food supplement formulations
- Natural colors and flavors
- Cosmetic products
- Ayurvedic preparations

Turmeric Extract- An Introduction

Turmeric (*Curcuma longa*) oldest nutrition and medicinal plant. 3000+ Publication, 1000+ studies confirm the therapeutic potential of turmeric. Application for 4000 years. Origin to the Indian soil and weather, India is the largest producer & exporter of Turmeric in the world

- Antioxidant
- Anti-inflammatory agent
- Inhibits oxidation of blood fats (serum lipid peroxides)
- Supports healthy immune system
- Inhibits platelet aggregation
- Protects the liver against toxins
- Lowers cholesterol

Turmeric Extract- Farming

- 100% Monitored cultivation & harvesting process
- Application of natural pesticides & fertilizers obtained from neem
- Traceability of cultivation region, cultivation process, harvesting details, seed variety & quality
- Dedicated facility for cleaning and sorting

Turmeric Extract- Processing

- Sorting between rhizomes and longitudinal fingers
- Washed and cleaned with sugar cane alcohol
- Sun dried
- Triple polished to remove scales & obtain smooth, clean turmeric rhizomes

Turmeric Extract- Manufacturing

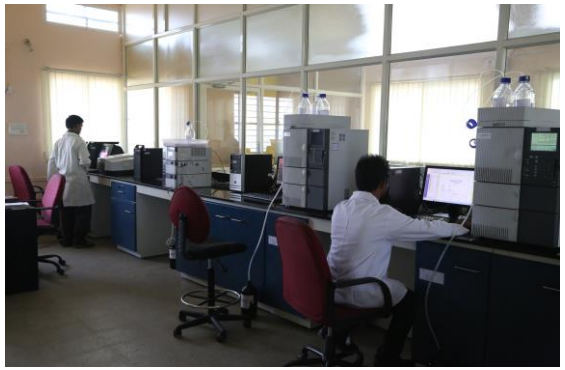
- Dedicated botanical extraction facility
- Use of raw material with highest content of curcuminoids for extraction
- Proprietary manufacturing process without use of harmful organic solvents
- Custom blends, granulation
- **FSSC 22000, NSF GMP, AYUSH GMP, ISO, KOSHER, HALAL Certified**

Turmeric Extract- Manufacturing Capacity

Continuous extraction capacity	20 TPD
Batch process extraction	25-30 TPD, Extractors-10 KL-10 N0, extract storage capacity-5000 liters x 10 N0
Size reduction	30-35 TPD Milling capacity
Volume reduction	RFE-3 N0, 1500 Liters/hr
Post extraction processing	Reactors-2KL-5N0, 1KL-3N0, 0.5 KL-2N0, Glass assembly 100 Liters-2 N0, Mixers, filtration,
Drying	Steam Heated Driers-50 tray 10 N0
Analytical & instrumentation	5000Sqft area, HPLC-3N0, GC-2 N0, UV Spectrometer-3N0
Storage	RM Storage-35,000 Sqft, Cold Storage-35,000Sqft
Solvent Storage	10,000 liters X 10 Under Ground tanks
Formulation & packing area	20,000 Sqft
QA & Process control, documentation	2500 Sqft, Documents, control sampls, in-process control

Turmeric Extract- Quality check

- Every lot tested for active principles
- Test for heavy metals and aflatoxins
- Test for solvent residue
- Test for microbial limit
- Stability data



Turmeric Extract- Product Specification

Parameter	Specification	Protocol#
Physical		
Description	Orange yellow* powder with characteristic odor	Organoleptic
Identification	To Comply by HPLC	USP 40
Solubility	Soluble in acetone Slightly soluble in alcohol Insoluble in water	USP
Loss on drying	Not more than 2.0% w/w	USP <731>
Ash content	Not more than 1.0% w/w	USP <281>
Untapped bulk density	Between 0.30 g/ml and 0.60 g/ml	USP <616>
Tapped bulk density	Between 0.50 g/ml and 0.80 g/ml	USP <616>
Particle size	100% passing through 40 mesh	USP <786>
Chemical		
Content of Total Curcuminoids by HPLC	Not less than 95.0% w/w	USP 40
Heavy Metals	ICP-OES	
Lead	Not more than 3.0 ppm	USP <233>Procedure1
Arsenic	Not more than 1.0 ppm	USP <233>Procedure1
Cadmium	Not more than 1.0 ppm	USP <233>Procedure1
Mercury	Not more than 0.1 ppm	USP <233>Procedure1
Microbiological		
Total plate count	Not more than 3000 cfu/g	USP <2021>
Total yeast and mold count	Not more than 100 cfu/g	USP <2021>
Escherichia coli	Negative / 10 g	USP <2022>
Salmonella spp.	Negative / 10 g	USP <2022>
Coliforms	Negative / 10 g	USP <2021>
Staphylococcus aureus	Negative / 10 g	USP <2022>
Pseudomonas aeruginosa	Negative / 10 g	USP <62>

Turmeric Extract- M F C

A

Curcuma longa rhizomes
Dry under sun. Pulverize through 8mm mesh

B

Curcuma longa rhizomes powder
Extract with alcohol for two hours (four times).
Filter through online filter (5µ) with magnetic trap.
Concentrate under vacuum.

C

Alcohol extract paste
Wash with alcohol, filter.
Dry the material under vacuum.
Pulverize, Sieve, Pack and Analyze.

D

Curcuma longa extract 95%

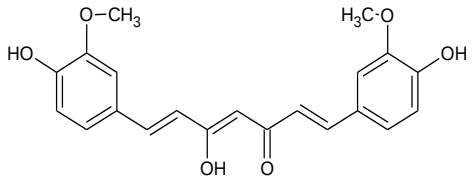
Turmeric Extract - Ingredient List

INGREDIENT(S)				
ACTIVES				
COMMON OR USUAL NAME	**SYNONYM**	**ORIGIN**	**CAS NUMBER**	**PERCENTAGE COMPOSITION**
Curcuma longa extract (Curcuminoids 95%)	Curcuma longa extract 95%	India	84775-52-0	100%
EXCIPIENTS/CARRIERS/ADDITIVES				
No excipients, carriers and additives are used				

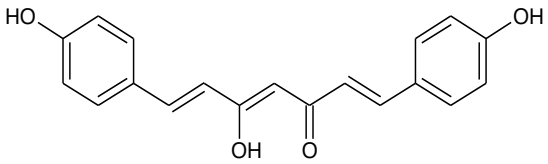
Turmeric Extract- Extract Structure

Phytoconstituents:

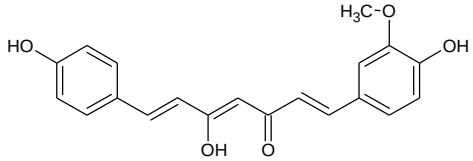
Curcumin, bisdemethoxycurcumin and demethoxycurcumin are altogether known as curcuminoids, is the principle bioactive of turmeric.[1] Tumerones (a and b), curdione, curzerenone, mono- and di-demethoxycurcumin have been reported from turmeric rhizomes.[2]



Curcumin



Bisdemethoxycurcumin



Demethoxycurcumin

Turmeric Extract – Efficacy /Benefits

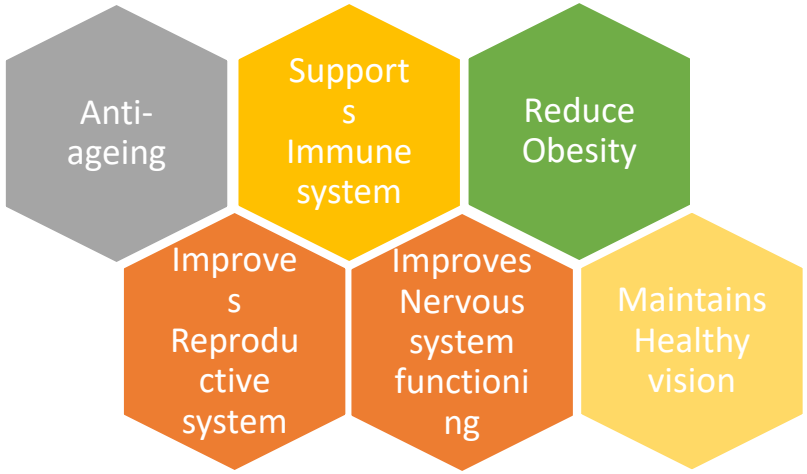
- The best antioxidant by nature
- Reduces cardiovascular complications.
- Improves brain & age related cognitive functions like Alzheimer.
- Helpful in managing pain & oxidative stress related complication in bone health.
- Works as lipid & Inflammation lowering in metabolic disease like diabetic and obesity, PCOS.
- Helpful in immunomodulation & inflammation controlling during Respiratory complications like asthma.
- Helpful in cancer treatment in managing oxidative stress, cancer cell proliferation.
- Helpful in managing the Prostate health.
- Helpful in managing the Liver health, suitable for soft gel capsules, creams etc.

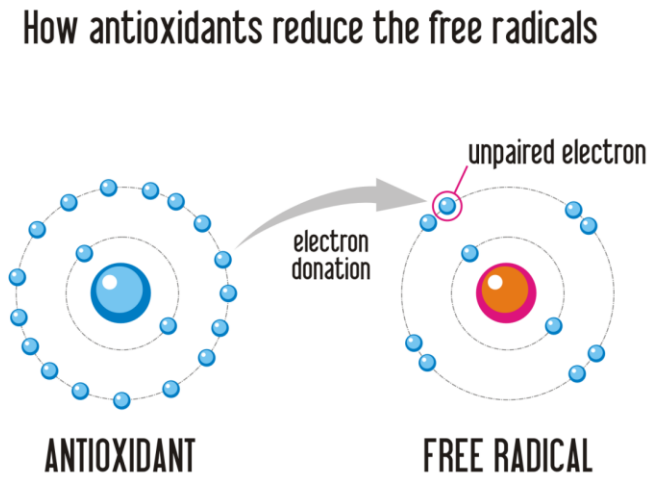
Turmeric Extract- Anti Oxidant

Clinical indication:

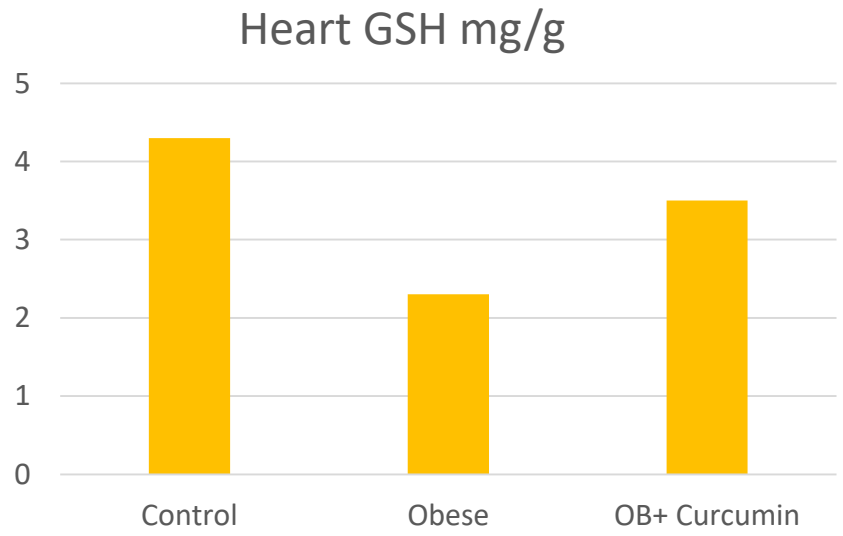
Curcumin has been reported to exhibit good antioxidant properties. Turmeric used traditionally for treatment of inflammation and skin wounds. Turmeric is widely used to treat biliary disorders, sinusitis, rheumatism, hepatic disorders, diabetic complications and inflammation. Curcuminoids inhibits platelet aggregation, reduces blood cholesterol, suppresses symptoms associated with type 2 diabetes, and prevents low density lipoprotein oxidation and rheumatoid arthritis and oxidative stress.[3]

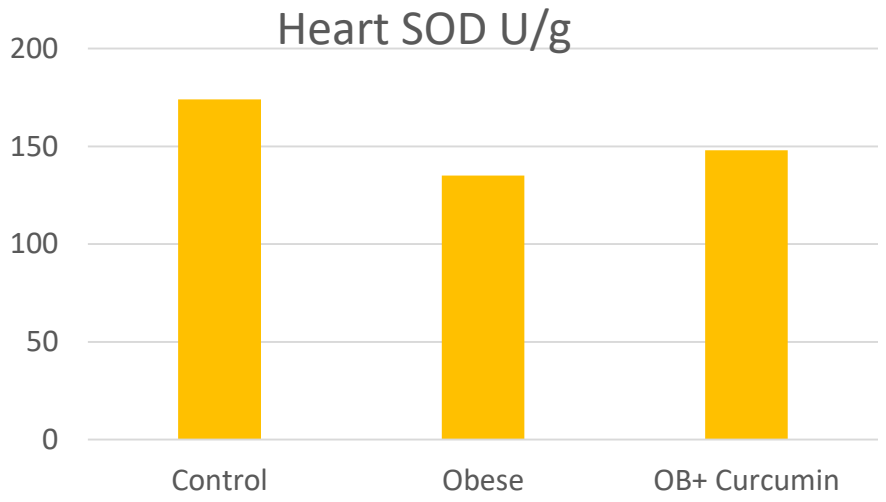
Antioxidants are substances which prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition.





Turmeric Extract- Anti Oxidant



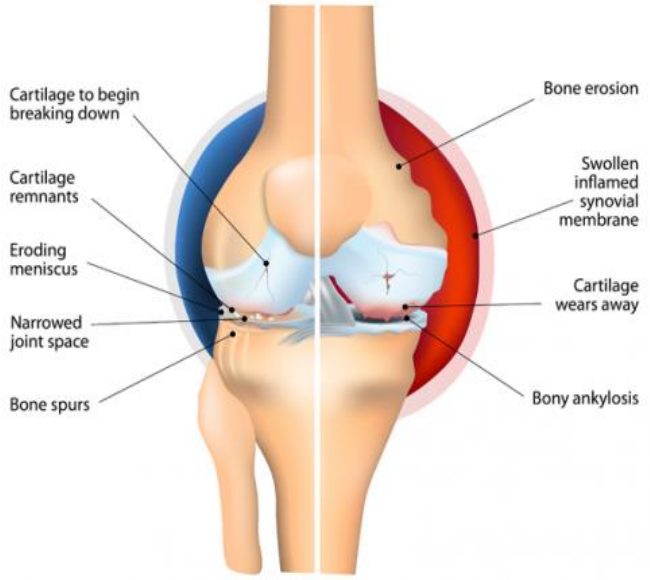


Endogenous anti-oxidants Levels are improved @ Heart –effect reduces oxidation of lipids @ heart- results in Improved blood circulation

Turmeric Extract- Anti Arthritis

Arthritis/ auto-immune disease improvement with Curcuminoids

Study plan
Animal: Rat, RA induced
Duration: 21 Days
Groups
Control:
CIA :
Rifampicin: 2.5mg/kg
Curcumin : 200mg/kg



Study result and summary:

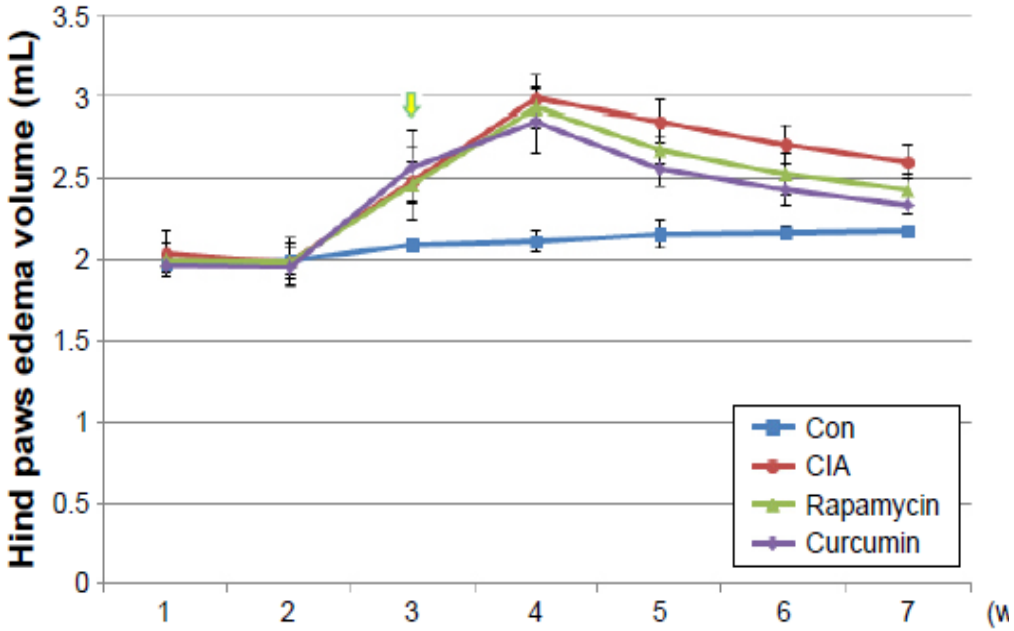
Curcumin inhibited the CIA-induced , RA-induced infiltration of inflammatory cells into the synovium. inhibited the increased levels of pro-inflammatory cytokines including IL-1β, TNF-α, MMP-1, and MMP-3 in CIA rats., pain and arthritis score

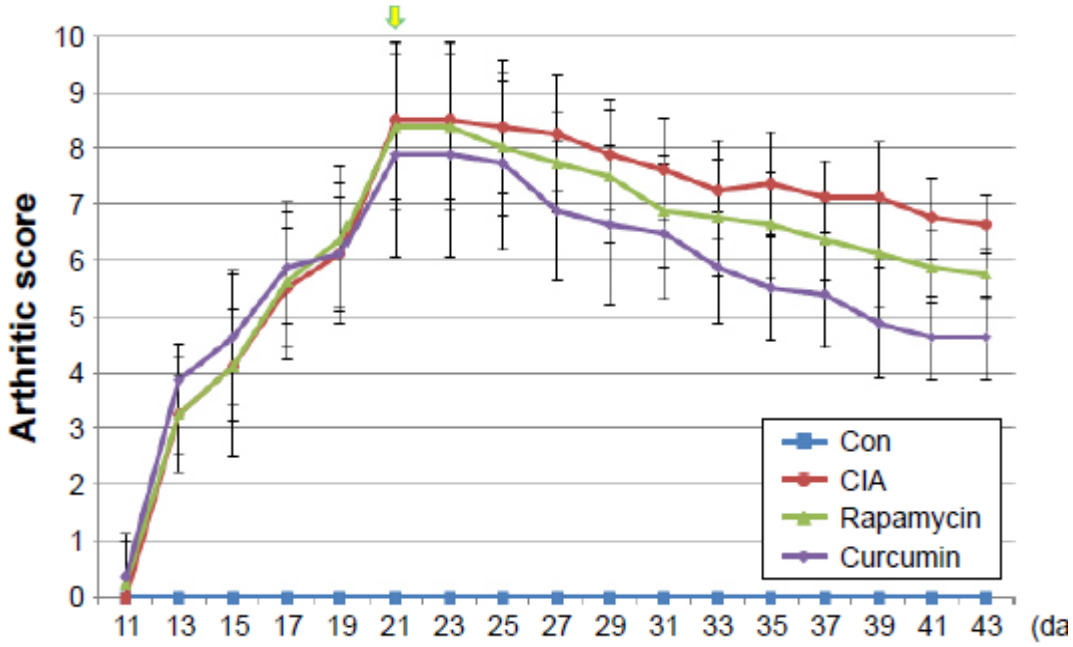
Drug Design, Development and Therapy 2018:12

Turmeric Extract- Anti Arthritis

Arthritis pain and score improved with curcuminoids

Drug Design, Development and Therapy 2018:12



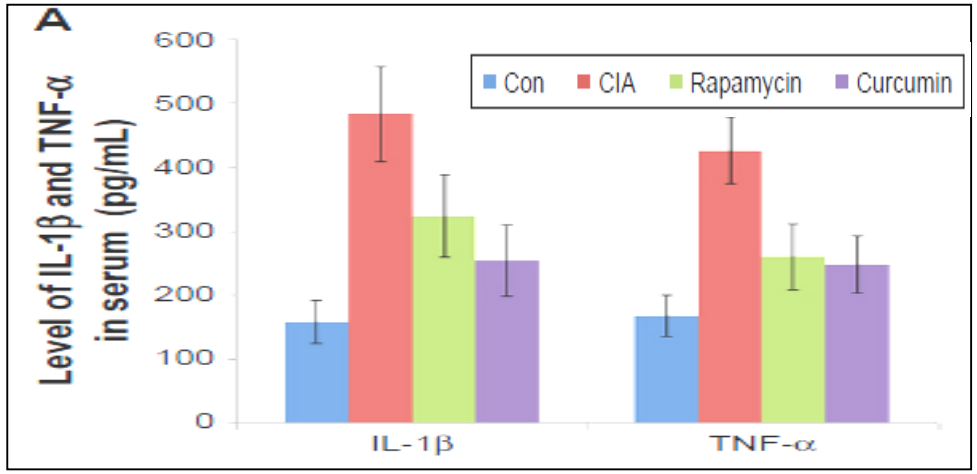


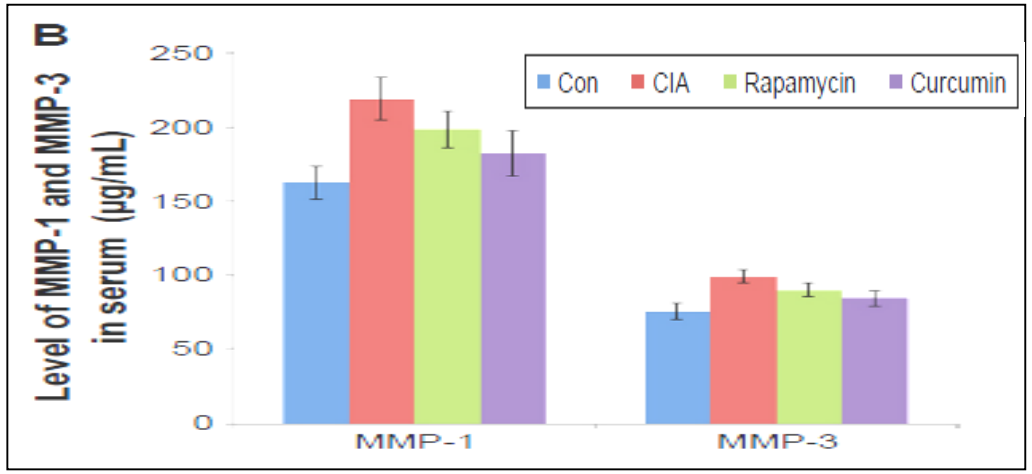
Arthritic Pain & score are reduced with curcumin

Turmeric Extract- Anti- Inflammation

Inflammation reduced, collagen degradation minimized with curcuminoids

Drug Design, Development and Therapy 2018:12





Inflammation markers TNF, IL-1, are decreased- resulting in reduced pain & degradation of bone, Collagen degrading enzymes are reduced – effecting the reduced bone loss and collagen building

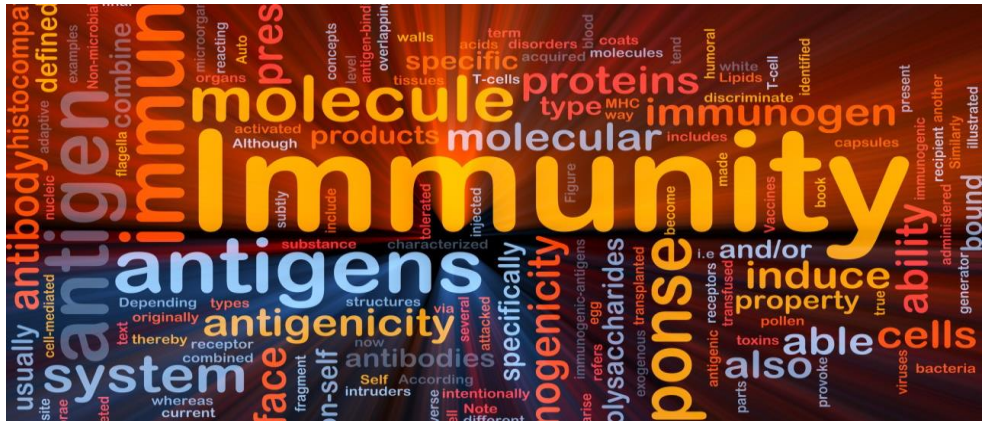
Turmeric Extract- Immunity Developer

Immune modulation/arthritis/autoimmune disease healing ability of curcuminoids

Indian Journal of Rheumatology ¦ Volume 12 ¦ Issue 2 ¦ June 2017 88

Study plan

Animal: Fifty female BALB/c mice, 6–8 weeks old
Duration: 16 Weeks
Groups
Normal:
Control:
Curcumin : 12.5mg/kg, 50mg/kg, 200mg/kg



Study result and summary:

The decreased of Th1, Th2, and Th17 percentages were seen after treatment with curcumin . Treatment decreased serum IL-6 and IFN-α levels. *Study concludes curcuminoids are able to modulate immune /inflammation immune related complications*

Bio Availability Study - BioTurmin™

BioTurmin™

- Trademarked by Olive Lifesciences.
- Enhanced Bioavailability.
- Pesticide free/ Organic - Certified
- Clinically proven.
- Clear water Dispersion.
- Natural Food Colorant.

Bio Availability Study - BioTurmin™

Bioavailability Studies of BioTurmin Water Dispersible Curcuminoids in Caco-2 Cell Model

BioTurmin™ Standardized extract of *Curcuma Longa* - Water dispersible

Bioavailability is defined as the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

Bio Availability Study - BioTurmin™

Introduction

What is Caco2 cell line?

The Caco-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells in intestine. it is developed by the Sloan-Kettering Institute for Cancer Research Caco2 cell line is differentiates spontaneously into enterocyte cell line.

Bio Availability Study - BioTurmin™

Intestinal Absorption: Biology

Enterocite biology:

Absorptive cells of intestine, function is terminal digestion and absorption of water and nutrients from the intestinal lumen.

Polarised monolayer :

Apical: microvilli face the interior of gut and increase the surface available for absorption by>1000 fold

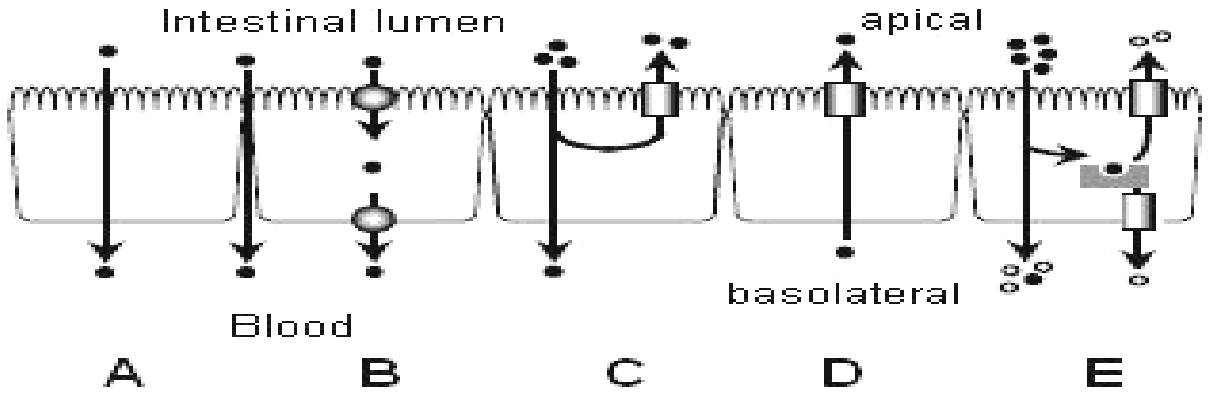
Basal: faces away from gut in contact with extracellular matrix.

Bio Availability Study - BioTurmin™

Intestinal transport mechanism: Major types

- Para cellular: For hydrophilic drugs with MW < 200
- Transcellular: For most lipophilic drugs. This route involves either passive diffusion, carrier mediated or receptor mediated endocytosis

Bio Availability Study - BioTurmin™



- **A:** passive trans- and paracellular diffusion;
- **B:** carrier mediated absorption at apical and basolateral membranes;
- **C:** active efflux transporter on apical membrane, acting during absorption;
- **D:** active efflux transporter on apical membrane, offering an additional route for drug clearance from the circulation;
- **E:** intracellular metabolising enzymes localized inside the enterocytes, possibly combined with an active efflux transporter on apical and basolateral membranes.

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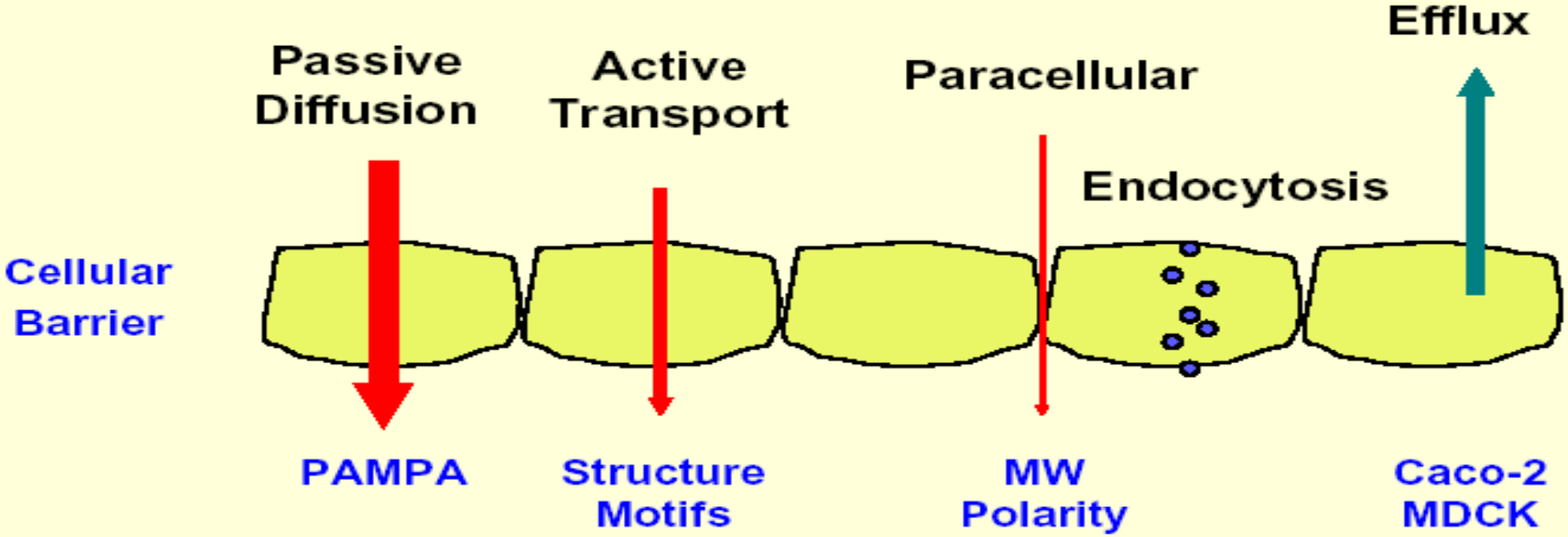

Passive diffusion: major absorption pathway

Li Di, *et al.*, Eur. J. Med. Chem., 2003, 38, 223-232

Edward Kerns - NIH-NIAID - 2-7-07

Bio Availability Study - BioTurmin™

Intestinal absorption models

1. Animal studies (Rat) - Very low throughput

2. *In situ* intestinal models - Very low throughput, expensive

Human/rat primary intestinal cells -Short functional life, lose differentiation characteristics

3. Intestinal Epithelial Barrier Models

- MDCK cell line: Madin-Darby Canine kidney cell line, varied transporter expression , in vitro model for BBB
- HT 29 Cells: Colon carcinoma, cultured with galactose, express mucus producing goblet cells differentiation
- Caco-2 Monolayer: Human colorectal adenocarcinoma Cell monolayer

Bio Availability Study - BioTurmin™

Caco-2 cell culture

- The Caco-2 cell line is an immortalized line of heterogeneous human epithelial colorectal adenocarcinoma cells, developed by the Sloan-Kettering Institute for Cancer Research.
- Caco-2 cell monolayers spontaneously differentiate to express morphological and functional characteristics of mature small-intestinal enterocytes. The differentiated monolayers are polarized, with microvilli on the apical side.

Bio Availability Study - BioTurmin™

Advantage of Caco-2 monolayer

- Spontaneously differentiate to express morphological (polarized columnar cells) and functional characteristics of mature small-intestinal enterocytes
- Four times higher in transepithelial resistance compared to HT 29-cell monolayer
- It expresses various drug metabolizing enzymes like, aminopeptidase, esterase, and sulfatase.

Bio Availability Study - BioTurmin™

Limitation of CaCO-2 monolayer

- Tissue in the villus contains more than one cell type
- Dose not produce the mucus and unstirred water Observed in the intestine
- No P-450 drug metabolizing enzyme activity has been reported
- Expensive method
- Time consuming as 21 days required for full cell differentiation
- The necessity of LC / MS or HPLC for quantitation
- Influence of P-gp is difficult to estimate

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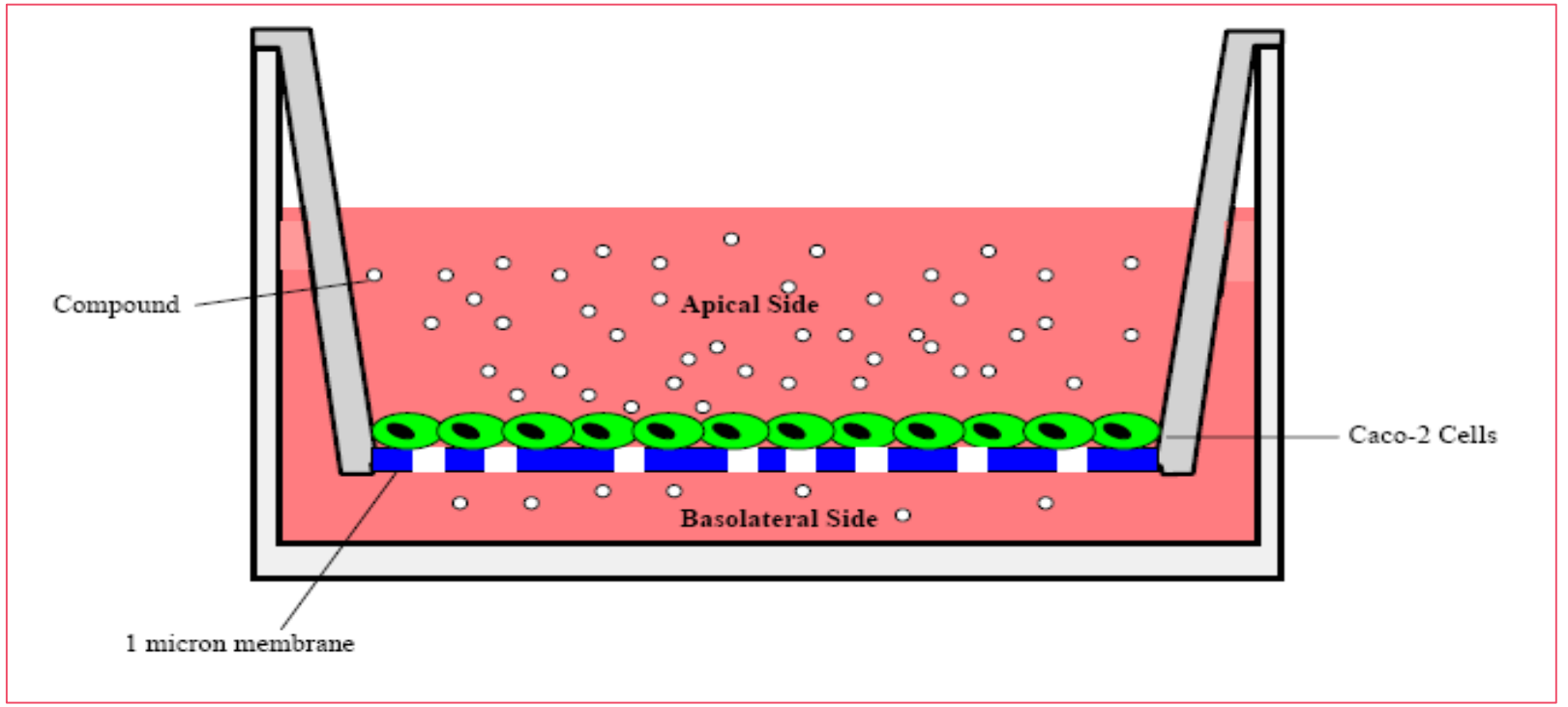
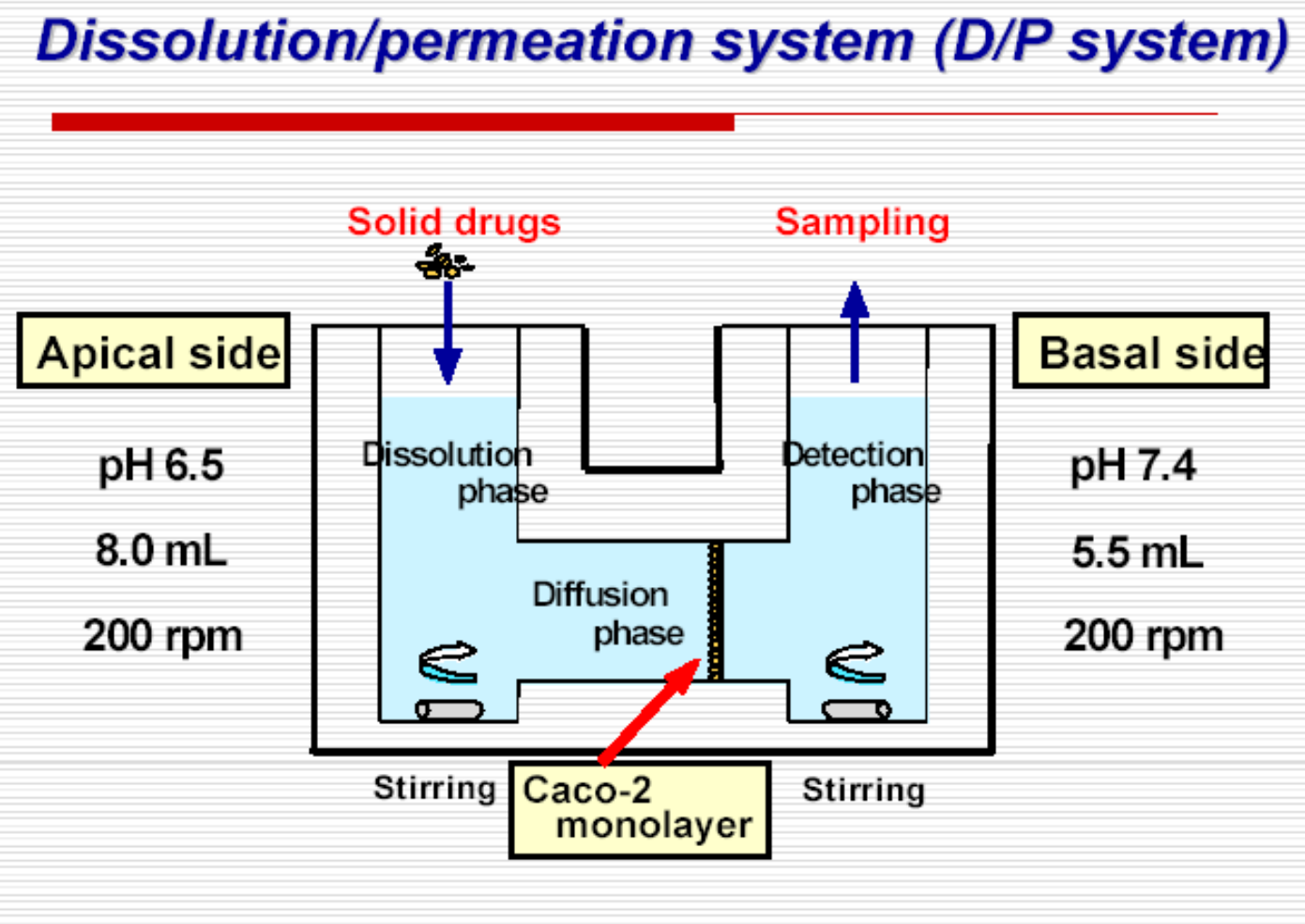
Biological pharmaceutical and analytical consideration

Assay Development



Bio Availability Study - BioTurmin™



Bio Availability Study - BioTurmin™

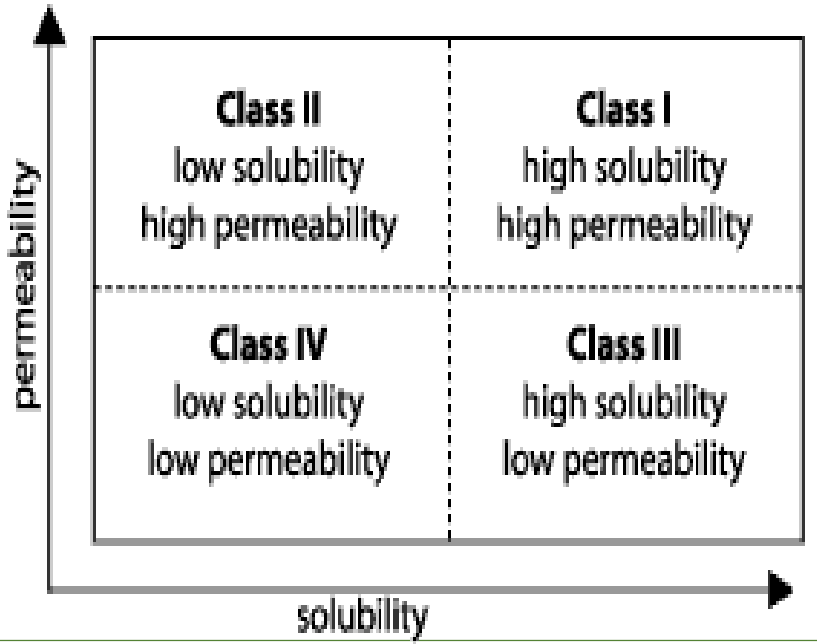
APPLICATION OF Caco-2 MODEL

- In Drug Discovery: To test the absorption profiles of the new molecular entities in the lead optimization state.
- In pre- clinical drug development: US FDA recognizes Caco-2 to measure permeability as part of *the bioequivalence waiver process.*
- To evaluate effect of pharmaceutical excipients.
- To study transport mechanism fro many compounds
- In drug metabolism & toxicity effects.
- Others like study of CFTR; regulation of protein expression; genetics study.

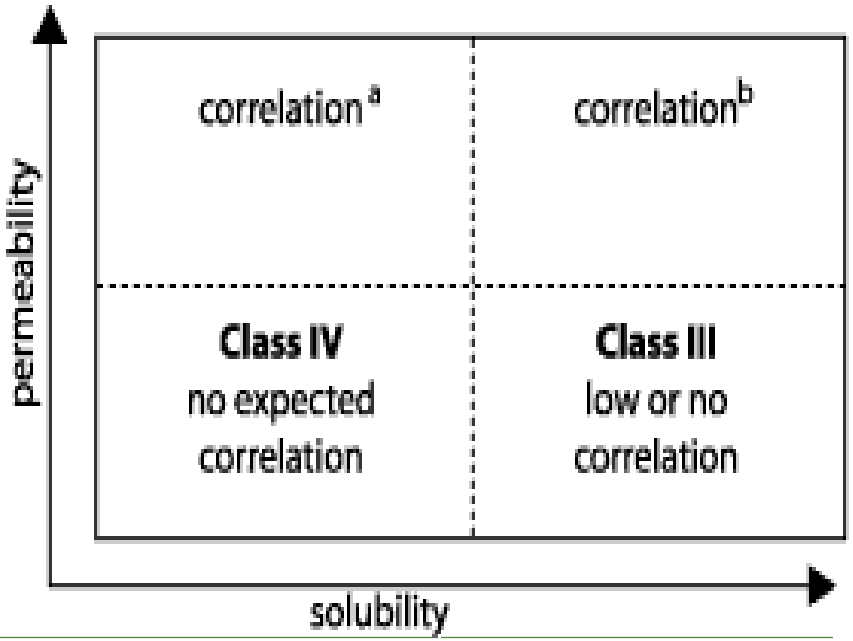
Bio Availability Study - BioTurmin™

CACO-2: PHARMACEUTICAL CONSIDERATIONS

Biopharmaceutical classification system



In vitro/in vivo correlation

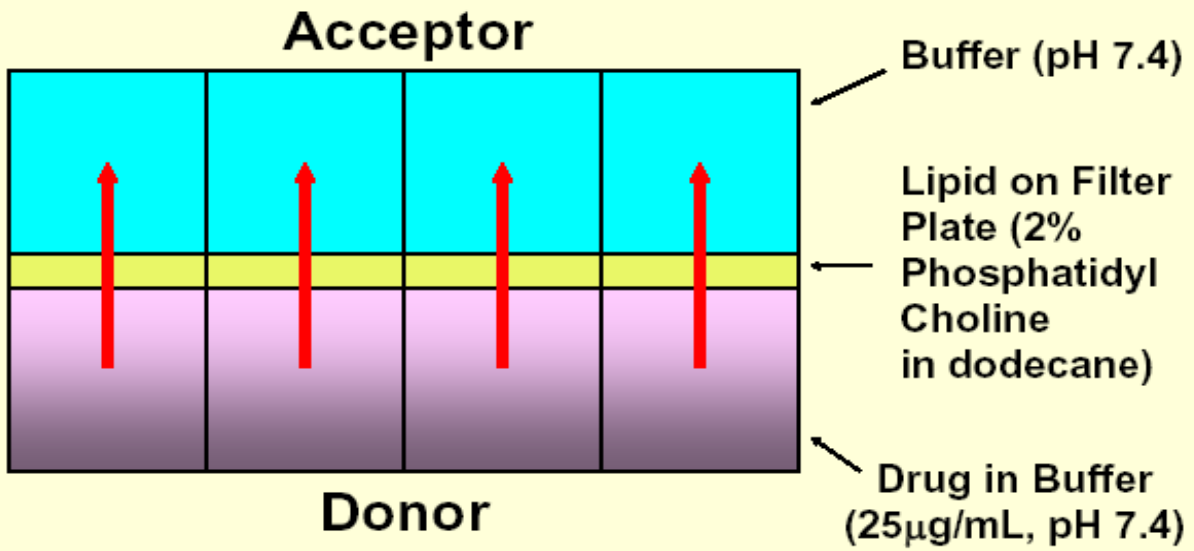


Bio Availability Study - BioTurmin™

Other model co related with caco2

PAMPA Method

"Parallel Artificial Membrane Permeability Assay"



Measures Passive Diffusion

Manfred Kansy, *et al., J Med Chem* (1998) 41, 1007

Edward Kerns - NIH-NIAID - 2-7-07

Wyeth Research

Bio Availability Study - BioTurmin™

PAMPA VERSUS Caco-2 MODEL

- PAMPA & caco-2 should not be considered as **competing permeability methods.**
- Good correlation between PAMPA & caco-2 data for a compound indicates a predominance of **passive diffusion in its permeation.**
- **Lack of correlation** indicates
- **Absorptive** (active, paracellular ,gradient effect for acids) or
- E**cretarys** (efflux, gradient effect for bases) permeation mechanism

Bio Availability Study - BioTurmin™

PAMPA MODEL	CACO-2 CELL MODEL
no cell culture involved, so not required long planning	based on cell culture, so required planning
High throughput & low cost	For HT, 96- well plats & compatible detection system
Useful only for passive transcellular permeability	For paracellular & transcellular permeability
Useful tool in early drug discovery to assess the permeability potential of large no. of compounds.	Method is more suitable during lead optimization or preclinical development stages, where true transepithelial permeability is needed.

Bio Availability Study - BioTurmin™

The Madin-Darby canine kidney (MDCK) cell model

- one of the commonly used cell monolayer systems to assess the human intestine barrier.
- MDCK cell lines can reach full differentiation in
- 3-7days and are therefore relatively easy for cell culturing and assay maintenance.
- DISADVANTAGE
- MDCK cell lines originate from dog kidney.
- The expression of transporters is quite different from human intestine.

Bio Availability Study - BioTurmin™

To compare the bioavailability of water dispersible curcuminoids (WDC) 5% (5% & 30%) with 95% pure curcuminoids through *in-vitro* drug permeability study in human Caco–2 cell line model.

Test Substance

Water Dispersible Curcuminoids (5% & 30%) were developed at

R & D Centre,

Olive Lifesciences Pvt. Ltd,

Tumkur, Karnataka, India.

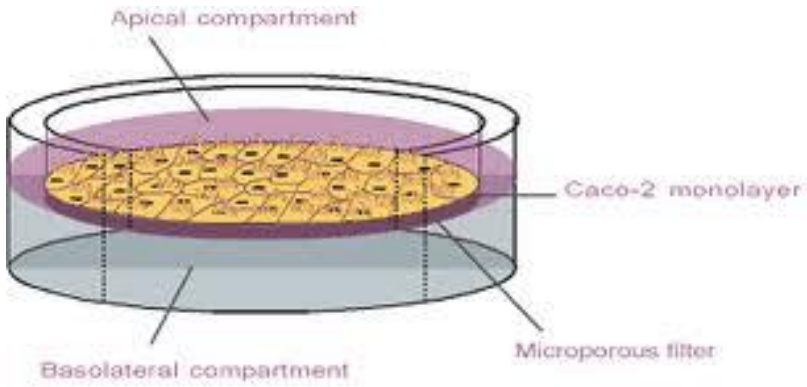
Bio Availability Study - BioTurmin™

Test Procedure

- Bioavailability study was carried out through dissolution profile followed by the uptake of Caco-2 cells.
- The doses of the test substance were confirmed based on the cytotoxicity study.
- Monolayer cultures of Caco-2 cells were trypsinized and seeded at 6 x 10^4 cells per cm^2 into the thin layer of thincert wells with 0.4 µm mean pore size and growth area of 1.13 cm^2 (12-wells).

500 µl of test solutions (20 µg/ml) dissolved in Hank's balanced salt solution (HBSS) were added to the apical side of the thincerts.

1500 µl of transport buffer was added to the basolateral side of each thincert. The plate was transferred to 37°C with 5% CO_2 humidity incubator.



Bio Availability Study - BioTurmin™

Test Procedure

- 100 µl samples were collected from basolateral side of the thincert and fresh transport buffer of equal volume was replaced at different time intervals i.e., 0.25, 0.5, 1, 2, 3, 4 and 24 hrs incubation.
- The sample solutions were further subjected to HPLC analysis for quantification of curcuminoids bioavailability.

Bio Availability Study - BioTurmin™

RESULTS

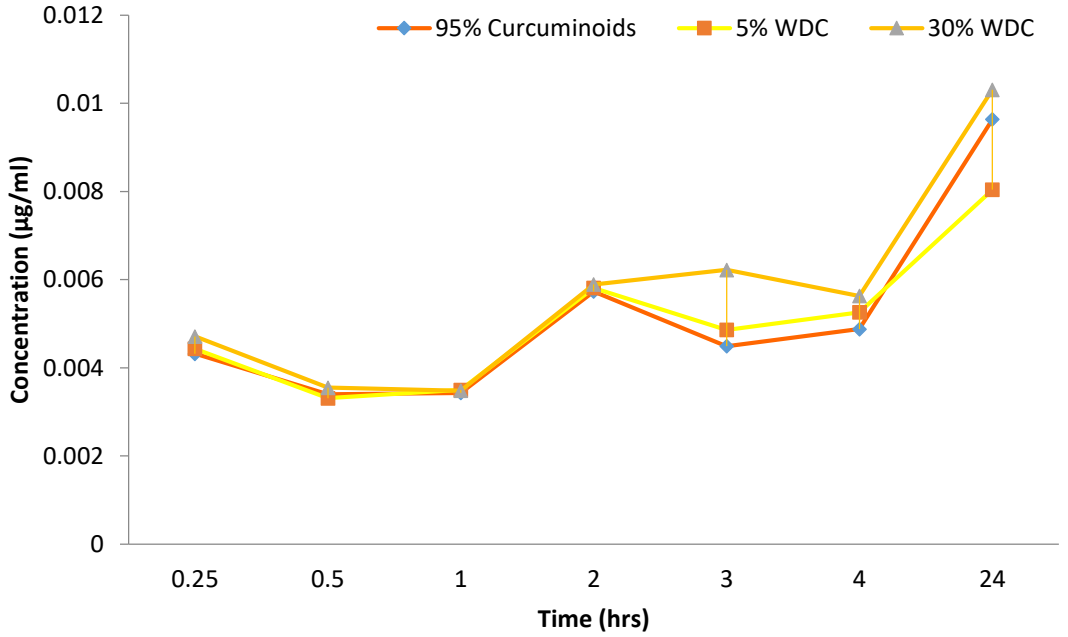
Cytotoxicity results against Caco-2 cells.

Sl. no.	Test sample	CTC_{50} (µg/ml)
1.	Water dispersible curcuminoids (5%)	21.54±0.1
2.	Water dispersible curcuminoids (30%)	38.01±0.1
3.	95% curcuminoids	>50

Bio Availability Study - BioTurmin™

RESULTS

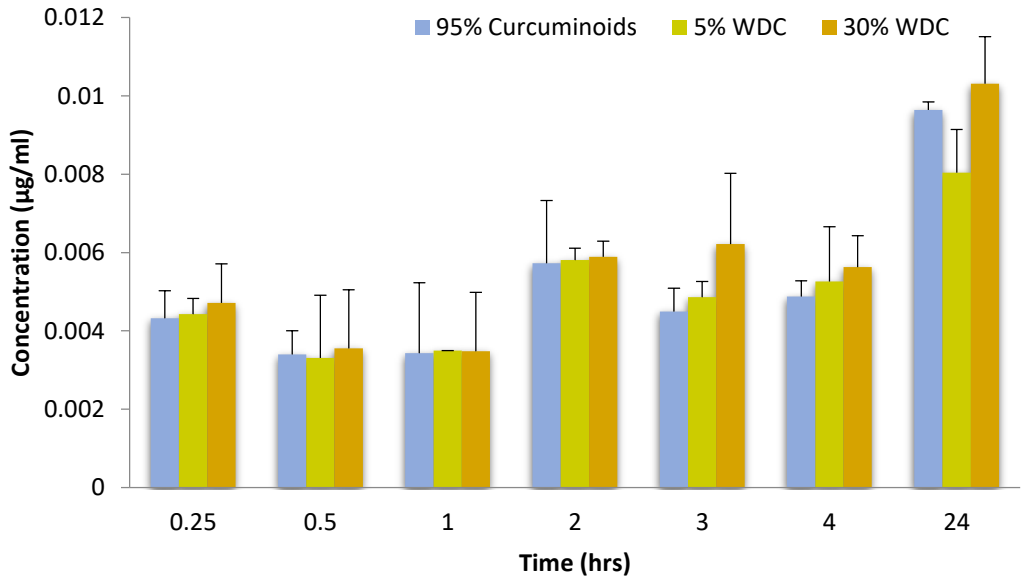
Bioavailability of 5% and 30% WDC formulations were nearly same compare to 95% pure standard curcuminoids up to 2 hrs



Bio Availability Study - BioTurmin™

RESULTS

During 2-24 hrs incubation period, there was increase in bioavailability of 30% WDC formulation compared to 5% WDC and 95% pure curcuminoids.



Bio Availability Study - BioTurmin™

CONCLUSION

Water dispersible curcuminoids (5% & 30%) showed better bioavailability than 95% curcuminoids.

Turmeric Extract - Dosage

Generally Recognized As Safe:

Turmeric is commonly used as spice in daily food preparation. In a human clinical trial study it has been recommended that up to 8000 mg/day dosages is safe to consume.[4] The U.S. Food and Drug Administration has approved curcumin as a “generally recognized as safe” (GRAS) substance and may be used as a food additive.[5]

Olive Lifesciences - A full spectrum value chain partner

We are a team committed to innovations and value creation through sustainable Agro Practices. We at Olive manufacture botanical extracts and phytochemicals for dietary supplements, food supplements, cosmeceuticals and food & beverage applications.

Our strength lies in our commitment across the value chain right from cultivation, extraction, formulation and value addition. We employ cutting-edge technology, innovation and an unflagging commitment to quality, to manufacture a wide range of products that comply with stringent global standards of plant operations, quality and safety.

The International quality standards have been certified by “FSSC 22000”, “ISO 9001:2008, “AYUSH”, Kosher, Halal & Organic USDA . The Research and development (R&D) center, certified by Department of Scientific & Industrial research (DSIR), Govt. of India, has developed some innovative and remarkable natural products.

Our journey has seen significant milestones and recognition. A Pharmexcil export award winner for 3 consecutive years, Shefexil export award, Outstanding Herbal Medicinal Plant Cultivator Award in recognition of Contribution in the field of conservation, cultivation and utilization of medicinal plants among several others.

Why To Choose Olive Lifesciences

Olive Lifesciences is privileged to offer a wide range of standardized botanical extracts, by adopting the GMP and cGMP guidelines, with source input materials being used in manufacturing are cultivated and collected as per GACP , by adopting cutting edge technology in manufacturing along with the support of high end phyto research and IQPA monitored QC and QA departments. We ensure the best, affordable, safe and efficaciously standardized extracts to each customer.

OVER 11 YEARS EXPERIENCE

We combine quality workmanship, superior knowledge and low prices to provide you with services unmatched by our competitors.

QUALITY STANDARDS

GMP certified facility, FSSC, HACCP, KOSHER, HALAL and ISO certified products, JAS certified raw materials, we are always a step ahead in quality.

DSIR APPROVED R & D

With DSIR approved Research facility we develop products that are completely customer oriented and as per market needs.

SEED To SHELF

OWN
CULTIVATION

Largest cultivator of Marigold, Turmeric , Coleus , Sesamin from INDIA

ECO FRIENDLY
PRODUCTION

Dedicated manufacturing facilities with world class quality certifications

TECHNICAL
SUPPORT

Qualified, well trained , experienced technical staff support till end product/ end user*

Quality Certifications

FSSC 22000

Standardized botanical extracts, by adopting the GMP and cGMP guidelines, with source input materials being used in manufacturing are cultivated and collected as per GACP , by adopting cutting edge technology in manufacturing along with the support of high end phyto research and IQPA monitored QC and QA departments.

HACCP CERTIFIED

Nutritional **Ingredients**

Natural **Flavors**

Natural **Colors**

Natural **Cosmetic**

Turmeric Extract - Our Grades

- Standardized Curcuminoids 95% - Regular powder
- Standardized Curcuminoids 95% - Granular
- Standardized Curcuminoids 85% - Ethanol extract
- BioTurmin – WD 5%
- BioTurmin – WD 10%

Thank You- Contact Us

CONTACT US
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BioTurmin™

References
Kulkarni, et al. 2012. Extraction and purification of curcuminoids from Turmeric (*Curcuma longa* L.) Int J of Pharmacol and Pharmaceutic Tech. 1(2): 81-84.
Yadav et al. 2013. Turmeric (*Curcuma longa* L.): A promising spice for phytochemical and pharmacological activities. Int J Green pharm. 85-89
Shivaprasad, et al., 2014. Bioavailability Studies of BioTurmin-WD (Water Dispersible Curcuminoids) Using Caco-2 Cell Model. J food res. 3(3): 158-163
Cheng et al. 2001. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 21:2895–2900.
U.S. Food and Drug Administration. Food Additive Status List. (2005). Trtrieved from http://www.cfsan.fda.gov/~dms/opa-appa.html.