CHAPTER-3

Literature Review
1. Current scenario of herbal research and market

The World Health Organization (WHO) has estimated around 4 billion people i.e. 80% of the world population, at the present are using herbal medicine for several aspects of primary health care. The world market for phytochemicals alone exceeds several billion dollars per year. It is estimated that the global trade in medicinal plants is US$ 800 million per year. The herbal market, inclusive of herbs and medicinal plants, in the USA, is estimated; at approximately US$ 1.6 billion p.a. China with exports of over 120,000 tonnes p.a., and India with some 32,000 tonnes p.a. dominate the international markets. The annual export of medicinal plants from India is valued at Rs. 1200 million. It is estimated that Europe, annually, imports about 400,000 tonnes of medicinal plants with an average market value of US$ 1 billion from Africa and Asia.

There are over 750,000 plants on earth. Relatively speaking, only a very few of the healing herbs have been studied scientifically. Of these, only about 6% have been screened for biologic activity, and a reported 15% have been evaluated phytochemically. In recent times, worldwide, there is an upsurge and interest among scientific institutions, biological research institutions in the use of medicinal plants, crude extracts or active ingredients to treat various ailments. Almost all of the current research validating herbal medicine has been done in Germany, Japan, China, India, Taiwan, and Russia. Today, rather than using a whole plant, pharmacologists identify, extract, isolate, and synthesize individual components to capture the active properties. In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and other substances that are important in supporting a particular herb’s medicinal properties. These elements also provide an important natural safeguard.

In India, over 2600 plant species have been considered useful in the traditional system of medicine like Ayurveda, Unani, Siddha, and Home remedies. Number of herbal drugs and their compositions are recommended for combating human ailments in the ancient texts as well as in modern medicine. The biogeographic position of India is unique which makes India a rich in all the three levels of biodiversity i.e. species diversity, genetic diversity and habitat diversity. A survey conducted by the All India Coordinated
Research Project on Ethnobiology (AICRPE) during the last decade recorded over 8000 species of wild plants used by the tribals and other traditional communities in India for treating various health problems. One of the ancient classics, “Charak Samhita” is the oldest text available on the complete treatment of diseases which specifies the use of hundreds of herbs in the complete treatment of diseases. The Ayurveda, whose history goes back to 500 B.C., is one of the ancient health care systems, which is a potential source of indigenous drugs. A large number of such herbs are mentioned in “Bhavprakash” as well as "Aryavidhya Kalanidhi". “Indian Materia Medica” also gives a large number of medicinal plants for the treatment of various diseases.

2. Herbs commonly used by Indian Ayurvedic therapeutic system as Psychotropic and Behavioral drugs

Psychotropic word explains itself as related to mental activity (Psycho+tropic), thus the drugs affecting the brain are termed as Psychotropic drugs where as all the CNS related diseases shows behavioral changes in the subjects. Ayurveda is the 5,000 year old holistic Indian art of healing and rejuvenation that is recently becoming widely available and popular in the West. A recent study in the "Journal of Social Behaviour and Personality," said that Ayurvedic purification, including body massage and an herbal enema, reduced free radicals in the blood, increased energy, improved digestion, and reduced symptoms of illness.

Some of the common plants which are used in India

1. **BADAMA** (*Prunus amygdalus*) - Used to increase mental energy and as a nerve tonic in amnesia.

2. **BRAHMI** (*Bacopa monnieri*, brambhi, thyme-leaved grariola) - Used as nerve tonic and sedative. Has been use to treat "insanity", depression and epilepsy. Seizure protection was shown comparable to benzodiazepines. Shows anti-psychotic activity similar to chlorpromazine. Sometimes used with other agents to make formulations.

3. **GARIJARA** (*Daucus carota*) – used as Nerve tonic in cognitive dysfunction and epilepsy.
4. **HARIDRA** (*Curcuma longa*, turmeric) - Dipression, Alzheimer’s disease, Parkinson’s disease, schizophrenia, epilepsy.\textsuperscript{15, 16, 17, 18}

5. **JATAMANSI** (*Nardostachys jatamansi*) - Used to treat nervousness, anxiety and insomnia.\textsuperscript{19}

6. **KUMKUMA** (*Crocus sativus*) - Antidepressant, Alzheimer’s disease.\textsuperscript{20, 21}

7. **KAPIKACHCHHA** (*Mucuna pruriens*) - Used in alzheimer’s, Parkinson.\textsuperscript{22}

8. **KARPOOR** (*Cinnamomum camphora*) - Used for nervousness.\textsuperscript{23}

9. **MARICHA** (*Piper nigrum*, Vellaja, Black Pepper) - Serotonin enhancer, cognitive, Parkinson, Alzheimer’s, and seizure.\textsuperscript{24, 25}

10. **MANDUKAPARNI** (*Centella asiatica*, brahmananduki, gotu kola, pennywort) - Used as a sedative, alterative, and anxiolytic.\textsuperscript{26}

11. **KAVA** (*Piper methysticum*, awa, kava-kava wurzel, kawa, kew, tonga, yagona, intoxicating pepper) - Natural relaxant and sleep aid, generalized anxiety, and social and specific phobias.\textsuperscript{27}

12. **SHANKAPUSHPI** (*Evolvulus alsinoides*) - Used for anxiety and memory loss.\textsuperscript{28}

13. **VACHA** (*Acorus calamus*, bach, sweet flag) - Used as a sedative, anxiolytic, sleeping aid, nervine and rejuvenator of the nervous system. It is also used to reduce fatigue and to improve memory.\textsuperscript{29}

14. **ASVAGANDHA** (*Withania somnifera*) - Used to enhance memory and lesson age-related cognitive deficits. It prevents tolerance and dependence with morphine.\textsuperscript{30, 31}

15. **YAVANI** (*Hyoscyamus niger*) - Chronic dementia, hysteria, sedation.\textsuperscript{32}

3. **Pharmacological Profile of selected plants and active phoconstituent β-carotene**

3.1 **Carrot** (*Daucus carota*) and its active constituent β-carotene

The carrot is a variable biennial plant, usually growing up to 1 m tall and flowering from June to August. The umbels are claret-colored or pale pink before they open, then bright white and rounded when in full flower, measuring 3–7 cm wide with a festoon of bracts beneath; finally, as they turn to seed, they contract and become concave like a bird's nest.
**Distribution:** native to temperate regions of Europe, southwest Asia and naturalized to North America and Australia.

**Classification**

- **Domain:** Eukaryota
- **Kingdom:** Plantae
- **Phylum:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Apiales
- **Family:** Apioideae
- **Genus:** Daucus
- **Species:** Daucus carota var. sativus

**Major chemical Constituents:** Carrots contain carotenes, especially alpha- and betacarotenes, vitamin A, and dietary fiber. Red carrots also contain lycopene.

![Chemical Structures](image)

*Figure 3.1 Major Phytoconstituents of Daucus carota*
Pharmacological Activity

Antioxidant activity: In several clinical trials, ingestion of carrot juice significantly increased carotenoid levels in lipoproteins and feces, but only slightly increased antioxidant capacity. A study did find that carrot juice significantly reduced oxidative base damage. It has been recommended that future antioxidant studies be randomized controlled trials instead of cross-over trials.

Estrogenic properties: In an in vitro study, active extracts/fractions/compounds from Daucus carota exhibited estrogenic activity.

Gastrointestinal effects: In two clinical trials, consumption of 19-33g of carrots per day increased gastrointestinal transit time, fiber intake, and fecal bulking/weight, dry matter, fiber, and protein. Another clinical trial that supplemented the subjects' diet with powdered carrot fiber found that it did not significantly modify colonic motor effects, other than to initiate high amplitude propagated contractions at a more distal location than the subjects' habitual diet. In a clinical trial in children, a carrot-rice based rehydration solution significantly decreased the duration of diarrhea, mean fecal output, and mean fluid intake compared to two conventional glucose-based rehydration solutions.

Immune effects: Watzl et al. conducted two clinical studies to assess the effect of dietary carotenoids from vegetable supplementation on immune function after a low-carotenoid diet. In the earlier study, diet supplementation was two weeks of tomato juice, followed by two weeks of carrot juice, then two weeks of spinach powder. The carrot juice did not affect immune function, a result that may have been skewed due to the previous two weeks of tomato juice consumption. In the later study, carrot juice consumption alone after a low-carotenoid diet did not affect immune system function.

Insulin effects: In a clinical trial of 10 healthy approximately 40 year-old males, consumption of 200g of processed and cooked carrots significantly lowered glucose and insulin/C-peptide responses.
Pharmacodynamics/Kinetics

Kurilich et al. conducted a pharmacokinetics study using purple carrots. The subjects consumed 250 g raw (463 mcM of anthocyanins: 400 mcM acylated, 63 mcM nonacylated), 250 g cooked (357 mcM of anthocyanins: 308.5 mcM acylated, 48.5 mcM nonacylated), and 500 g cooked (714 mcM of anthocyanins: 617 mcM acylated, 97 mcM nonacylated) carrots. According to the authors, four of the five carrot anthocyanins were found intact in plasma 30 minutes after carrot consumption and peaked between 1.5 and 2.5 hours. Acylation of anthocyanins resulted in an 11- to 14-fold decrease in anthocyanin recovery in urine and an 8- to 10-fold decrease in anthocyanin recovery in plasma. Cooking increased the recovery of nonacylated anthocyanins but not acylated anthocyanins. Large dose size significantly reduced recovery of both acylated and nonacylated anthocyanins, suggesting saturation of absorption mechanisms.

In another clinical trial, Thurmann et al. investigated the bioavailability of beta-carotene from carrot juice and a water dispersible beta-carotene powder. According to the authors, apparent steady-state beta-carotene concentrations were attained after 40 days of supplementation. Consumption of the beverage containing beta-carotene powder resulted in a higher response of beta-carotene plasma concentrations with increments of $3.84 \pm 0.60$ mcM/L ($p<0.05$, dose: 7.2 mg per day) and $5.04 \pm 0.72$ mcM/L ($p<0.05$, dose: 21.6 mg per day), respectively, in comparison to the carrot juice-based drink with increments of $0.42 \pm 0.33$ mcM/L (dose: 6 mg per day) and $1.71 \pm 0.55$ mcM/L (dose: 18 mg per day), respectively. Beta-carotene was cleared from the plasma with an apparent half-life of six to 11 days. Plasma concentrations of alpha-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and lycopene remained almost unchanged, whereas retinol plasma concentrations increased slightly. By contrast, with the exception of elevated 13-cis-retinoic acid in one group (21.6 mg per day, water dispersible powder), the concentrations of all-trans-retinoic acid and the oxo-derivatives or retinoic acid were not significantly affected by beta-carotene supplementation.

Horovitz et al. conducted a bioavailability study of the lycopene in red carrots. In the first of two cross-over studies, subjects (n=9) ingested muffins containing white carrots (0 mg...
lycopene per day), red carrots (5 mg per day), and tomato paste (20 mg per day). Study 2 (n=10) used muffins containing red carrots (2.6 mg per day), tomato paste (5 mg per day), and tomato paste plus white carrots (5 mg per day). Each intervention lasted 11 days with a 10-day washout period between treatments. According to the authors, statistical analysis indicated a significant effect of muffin type in study 1 (p<0.001), and a significant treatment by sequence interaction in study 2 (p=0.04). The response to increasing amounts of lycopene is linear at the levels fed in these studies (r=0.94). The data suggest that maintenance of serum lycopene concentrations at 0.3 mcM/L occurs at about 2mg per day of lycopene from mixed dietary sources and a serum plateau occurs at ≥20 mg per day.47

Cardinault et al.48 conducted a study to test the effect of age on carotenoid bioavailability. According to the authors, eight young (20-35 years) and eight older (60-75 years) healthy adults ingested three different meals containing 40 g triacylglycerols and vegetable sources of carotenoids. These sources were either 188 g carrot puree (30mg beta-carotene), or 61 g tomato puree (30mg lycopene), or 260 g cooked chopped spinach (30mg lutein). Triacylglycerols and carotenoids were assayed in chylomicrons collected for nine hours postprandially. There was no major effect of age on the postprandial chylomicron: triacylglycerol response (0-9 hour area under the curve (AUC)). There was no major effect of age on the postprandial chylomicrons all-trans beta-carotene, cis beta-carotene, alpha-carotene, and lutein responses. Adjustment of these responses by the chylomicron: triacylglycerol responses did not reveal any age effect. While there was no significant effect of age on the chylomicron: lycopene response, the chylomicron: triacylglycerol-adjusted lycopene response was significantly lower (-40%) in the older than in the younger subjects (p<0.04). The cis-trans ratios of chylomicron: β-carotene were not significantly different between the old and the young subjects. There was no significant effect of age on the ratio of chylomicrons: retinyl-palmitate to the sum of alpha-carotene and beta-carotene measured after the carrot meal.

Tyssandier et al. conducted another study to assess the intestinal absorption of carotenoids. According to the authors, 10 healthy men were intragastrically fed three liquid test meals differing only in the vegetable added three weeks apart and in a random order. They
contained 40 g sunflower oil and mashed vegetables as the sole source of carotenoids. Tomato puree provided 10 mg lycopene, chopped spinach (10 mg lutein), and carrot puree (10 mg beta-carotene). All-trans and cis carotenoids were assayed in stomach and duodenal contents, in the fat and aqueous phases of those contents, and in chylomicrons. The cis-trans beta-carotene and lycopene ratios did not significantly vary in the stomach during digestion. Carotenoids were recovered in the fat phase present in the stomach during digestion. The proportion of all-trans carotenoids found in the micellar phase of the duodenum was as follows (means ± SE): lutein (5.6 ± 0.4%), beta-carotene (4.7 ± 0.3%), and lycopene (2.0 ± 0.2%). The proportion of 13-cis beta-carotene in the micellar phase was significantly higher (14.8 ± 1.6%) than that of the all-trans isomer (4.7 ± 0.3%). There was no significant variation in chylomicron lycopene after the tomato meal; however, there was significant increase in chylomicron beta-carotene and lutein after the carrot and the spinach meals, respectively. There is no significant cis-trans isomerization of beta-carotene and lycopene in the human stomach.49

Kirkman et al. compared the urinary lignan and isoflavonoid excretion in 11 men and nine women consuming four nine-day controlled experimental diets: basal (vegetable free), carotenoid vegetable (carrot and spinach), cruciferous vegetable (broccoli and cauliflower), and soy (tofu and textured vegetable protein product). According to the authors, three-day urine collections (days 7-9) were analyzed for lignans and isoflavonoids with use of isotope-dilution gas chromatography-mass spectrometry. Urinary excretion of the lignans enterodiol and enterolactone was higher in the carotenoid and cruciferous vegetable diets than in the basal diet (p=0.0001), suggesting that these vegetables may provide a source of mammalian lignan precursors. Urinary excretion of the isoflavonoids equol, O-desmethylandgolensin, daidzein, and genistein was higher when subjects consumed soy diets than when they consumed the other test diets (p<0.02). Gender differences in lignan excretion were observed. Men excreted more enterolactone (p=0.006) and less enterodiol (p=0.013) than women, implying a gender difference in colonic bacterial metabolism of lignans. There was no effect of gender on isoflavonoid excretion.50
In a pharmacokinetics study, Agte et al. compared consumption of micronutrients through vegetables to consumption of micronutrients through tablet supplementation. The study was a short-term (0-4 hour) response (area under the curve, AUC) of single dose of 7.9 mg beta-carotene and 130 mg ascorbic acid through a spinach-carrot meal against the standard meal without green leafy vegetables plus 10 mg beta-carotene and 150 mg ascorbic acid tablets. There were two groups of four young volunteers each. The authors report in the second trial of three weeks' supplementation, five groups of young adults (n=40) were given either 100 g green leafy vegetables daily alone or with tablets of vitamin E (100 mg daily) or C (100 mg daily) or more oil (5 g daily) or non-green leafy vegetables meal with tablet of beta-carotene (10 mg daily). According to the authors, hemoglobin, plasma beta-carotene, zinc, vitamin C, glucose, and triglycerides were measured. In a postprandial response, AUC were comparable in both green leafy vegetables and standard meals for beta-carotene and ascorbic acid. In terms of triglycerides and glucose AUC, the green leafy vegetables meal showed a better recovery to the baseline value after four hours than the standard meal. Three weeks' supplementation of green leafy vegetables with more oil resulted in significant increase of plasma beta-carotene (51%) and hemoglobin (9%). Green leafy vegetables with vitamin E showed a significant increase in plasma beta-carotene (40%), hemoglobin (8%), and plasma vitamin C (6%). Supplementing beta-carotene without green leafy vegetables significantly increased hemoglobin (11%) and plasma zinc (14%) in addition to beta-carotene. Multiple regression analyses weighted for energy intake indicated a significant association of percent increase in hemoglobin with intakes of iron, riboflavin, folic acid, beta-carotene, copper, phytate, and fiber (p<0.01), percent change in plasma zinc with intakes of zinc, beta-carotene, vitamin C, riboflavin, copper, iron, and thiamin (p<0.01), percent change in vitamin C with intakes of vitamin C, vitamin E, niacin, riboflavin, thiamin, beta-carotene, zinc, phytate, and fiber (p<0.05), and percent change in plasma beta-carotene with intakes of beta-carotene, thiamin, folic acid, zinc, phytate, and tannins (p<0.05).\(^{51}\)
3.2 Black Pepper (*Piper nigrum*) and its active constituent Piperine

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. The fruit, known as a peppercorn when dried, is approximately 5 millimetres (0.20 in) in diameter, dark red when fully mature, and, like all drupes, contains a single seed. Peppercorns, and the ground pepper derived from them, may be described simply as pepper, or more precisely as black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit) and white pepper (unripe fruit seeds).

**Distribution:** Black pepper is native to south India, and is extensively cultivated there and elsewhere in tropical regions. Currently Vietnam is the world's largest producer and exporter of pepper, producing 34% of the world's *Piper nigrum* crop as of 2008.

**Classification**

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Superdivision: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Magnoliidae
- Order: Piperales
- Family: Piperaceae
- Genus: *Piper*
- Species: *nigrum*

**Major chemical constituents:** The fruit contains a large number of alkaloids and related compounds, the most abundant of which is piperine, together with piperlongumine, pipernonaline, piperlonguminine, tetrahydro piperine.
Pharmacological activity

**Acetylcholinesterase inhibitory activity:** In an *in vitro* study, an extract of *Piper nigrum* L. seeds showed 50-65% inhibitory activity on acetylcholinesterase.52

**Antibacterial effects:** In an *in vitro* study using 12 different genera of bacterial populations isolated from the oral cavity of 200 individuals, an aqueous decoction of black pepper (*Piper nigrum* L.) exhibited 75% antibacterial activity as compared to aqueous decoction of bay leaf (53.4%) and aqueous decoction of aniseed (18.1%), at the concentration of 10mL/disc.53

**Anti-inflammatory effects:** Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica, Terminalia chebula, Terminalia bellerica, Albizia lebbeck, Piper nigrum, Zingiber officinale*, and *Piper longum* demonstrated 31.3% inhibition against carrageenan-induced acute inflammation in Wistar Albino rats, while ibuprofen (50
mg/kg orally) exerted 68.1% inhibition.\textsuperscript{54} Aller-7 also exhibited a dose-dependent (150-350mg/kg) anti-inflammatory effect against Freund's adjuvant-induced arthritis in Wistar Albino rats; an approximately 63% inhibitory effect was observed at a dose of 350mg/kg.

**Antilarval activity:** Piptigrine, isolated from the dried ground seeds of *Piper nigrum* Linn., exhibited toxicity of 15.0ppm against fourth instar larvae of *Aedes aegypti* Liston.\textsuperscript{55}

**Antioxidant effects:** Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica*, *Albizia lebbeck*, *Piper nigrum*, *Zingiber officinale*, and *Piper longum* exhibited concentration-dependent scavenging activities toward biochemically generated hydroxyl radicals (IC\textsubscript{50} 741.73 mcg/mL); superoxide anion (IC\textsubscript{50} 24.65 mcg/mL by phenazine methosulfate-nicotinamide adenine dinucleotide [PMS-NADH] assay and IC\textsubscript{50} 4.27 mcg/mL by riboflavin/nitroblue tetrazolium [NBT] light assay), nitric oxide (IC\textsubscript{50} 16.34 mcg/mL); 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical (IC\textsubscript{50} 5.62 mcg/mL); and 2,2-azinobis-ethyl-benzothiozoline-sulphonic acid diammonium salt (ABTS) radical (IC\textsubscript{50} 7.35 mcg/mL). Aller-7 inhibited free radical-induced hemolysis in the concentration range of 20-80 mcg/mL. Aller-7 also significantly inhibited nitric oxide release from lipopolysaccharide-stimulated murine macrophages.\textsuperscript{56}

**Cytochrome P (CYP) 450 effects:** In *in vitro* studies, constituents isolated from *Piper nigrum*, including piperine and dipiperamides D and E, potently inhibited some CYP450 metabolic pathways, including CYP2D6 and CYP3A4.\textsuperscript{57}

**Gastrointestinal effects:** In a clinical study of intestinal peristalsis in 16 healthy volunteers, consumption of 1.5g of black pepper in capsules increased the orocecal transit time from 90 ± 51 minutes to 122 ± 88 minutes (p=0.09).\textsuperscript{58} In an *in vitro* study, piperine inhibited digoxin and cyclosporine A transport in Caco-2 cells with IC\textsubscript{50} values of 15.5 and 74.1mcM, respectively. The bactericidal and anti-adhesive properties of black pepper have also been investigated against *Helicobacter pylori*, however, aqueous extracts did not show bactericidal effect on any of the isolates.\textsuperscript{59}
**Neural effects:** In an *in vitro* study using whole-cell patch-clamp electrophysiology, piperine, a pungent alkaloid found in black pepper, had similar agonist effects on the human vanilloid receptor TRPV1 as capsaicin. However, piperine could induce greater receptor desensitization and exhibit a greater efficacy than capsaicin.  

**Pharmacodynamics/Kinetics**

**Pharmacokinetics:** In an animal study, piperine from black pepper was shown to enhance the bioavailability of EGCG, a polyphenol constituent from green tea (*Camellia sinensis*). Intragastric coadministration of 163.8 mcM/kg EGCG and 70.2 mcM/kg piperine to male CF-1 mice increased the plasma $C_{\text{max}}$ and area under the curve (AUC) by 1.3-fold compared to mice treated with EGCG only. The authors report that piperine appeared to increase EGCG bioavailability by inhibiting glucuronidation and gastrointestinal transit. Piperine (100 mM/L) inhibited EGCG glucuronidation in mouse small intestine (by 40%), but not in hepatic microsomes. Piperine (20 mcM/L) also inhibited production of EGCG-3"-glucuronide in human HT-29 colon adenocarcinoma cells. Small intestinal EGCG levels in CF-1 mice following treatment with EGCG alone had a $C_{\text{max}} = 37.50 \pm 22.50$ nM/g at 60 min that then decreased to $5.14 \pm 1.65$ nM/g at 90 min; however, cotreatment with piperine resulted in a $C_{\text{max}} = 31.60 \pm 15.08$ nM/g at 90 min, and levels were maintained above 20 nM/g until 180 min. This resulted in a significant increase in the small intestine EGCG AUC ($4,621.80 \pm 1,958.72$ vs. $1,686.50 \pm 757.07$ (nM/g·min)). EGCG appearance in the colon and the feces of piperine-cotreated mice was slower than in mice treated with EGCG alone.

**Pharmacodynamics:** Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica*, *Albizia lebbeck*, *Piper nigrum*, *Zingiber officinale*, and *Piper longum* exhibited concentration-dependent scavenging activities toward biochemically generated hydroxyl radicals ($IC_{50} 741.73$ mcg/mL), superoxide anion ($IC_{50} 24.65$ mcg/mL by phenazine methosulfate-nicotinamide adenine dinucleotide [PMS-NADH] assay and $IC_{50} 4.27$mcg/mL by riboflavin/nitroblue tetrazolium [NBT] light assay), nitric oxide ($IC_{50} 16.34$mcg/mL), 1,1-diphenyl-2-picryl hydrazyl (DPPH)
radical (IC$_{50}$ 5.62 mcg/mL); and 2,2-azinobis-ethyl-benzothiazoline-sulphonic acid diammonium salt (ABTS) radical (IC$_{50}$ 7.35 mcg/mL). In an *in vitro* study, piperine inhibited digoxin and cyclosporine A transport in Caco-2 cells with IC$_{50}$ values of 15.5 and 74.1 mcM, respectively.

### 3.3 *Curcuma longa* and its active phytoconstituents Curcumin

*Curcuma Longa/Cúrcuma* is a small perennial herb native to India bearing many rhizomes on its root system which are the source of its culinary spice known as Turmeric and its medicinal extract called Curcumin.

**Distribution:**

India (especially Andhra Pradesh and Tamil Nadu): 600,000 tons annually and Southeast Asia

**Classification:**

Kingdom: Plantae  
Subkingdom: Tracheobionta  
Superdivision: Spermatophyta  
Division: Magnoliophyta  
Class: Liliopsida  
Subclass: Zingiberidae  
Order: Zingiberales  
Family: Zingiberaceae - Ginger family  
Genus: *Curcuma*  
Species: *longa*

**Chemical constituents:**

The bright yellow color of turmeric is primarily due to fat-soluble, polyphenolic pigments known as curcuminooids, primarily curcumin (diferuloyl methane), dimethoxycurcumin and bis dimethoxycurcumin, and 2,5-xylenol.
Figure 3. 3 Major phytoconstituents of *Curcuma longa*

**Pharmacological action**

**Alzheimer's effects**: Beta-Amyloid (betaA)-induced oxidative stress is a well-established pathway of neuronal cell death in Alzheimer's disease. Three curcuminoids from turmeric (*Curcuma longa* L.), including curcumin, demethoxycurcumin, and bisdemethoxycurcumin, were found to protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial (HUVEC) cells from beta A(1-42) insult. These compounds may protect the cells from beta A (1-42) insult through antioxidant pathways. Other animal studies of Alzheimer's disease also suggest that curcumin may reduce levels of amyloid and oxidized proteins and prevent cognitive deficits. One alternative mechanism of action for these effects suggested by Baum et al. is metal chelation, which may reduce amyloid aggregation or oxidative neurotoxicity. Since curcumin more readily binds the redox-active metals and than the redox-inactive, curcumin might exert a net protective effect against beta toxicity or might suppress inflammatory damage by preventing metal induction of NF-kappaB. Mouse studies that evaluated the effects of dietary curcumin on inflammation, oxidative damage, and plaque pathology demonstrated that both low and high doses of curcumin significantly lowered...
oxidized proteins and interleukin-1beta, which is a proinflammatory cytokine elevated in the brains of these mice. Low-dose but not high-dose curcumin treatment has been shown to reduce the astrocytic marker GFAP and significantly decrease insoluble beta-amyloid (Abeta), soluble Abeta, and plaque burden by 43-50%. However, levels of amyloid precursor (APP) in the membrane fraction were not reduced.65

**Antibacterial effects:** The ethyl acetate extract of *Curcuma longa* L. has demonstrated a higher antibacterial activity than the methanol extract or water extract.66

**Anti-inflammatory effects:** Turmeric has been associated with the inhibition of tumor necrosis factor-α, interleukin-8, monocyte inflammatory protein-1, interleukin-1B, and monocyte chemotactic protein-1.67 Turmeric and its constituent curcumin have been found to inhibit lipoxygenase and cyclooxygenase in rat tissues and in vitro68, as well as thromboxane B2 and leukotriene B4 formation.69 Based on animal study, oral administration of curcumin may reduce expression of several cytokines, chemokines, and proteinases known to mediate aneurismal degeneration.70 In rat macrophages, curcumin inhibits the incorporation of arachidonic acid into membrane lipids, as well as prostaglandin E2, leukotriene B4, and leukotriene C4, but does not affect the release of arachidonic acid.71 Curcumin also inhibits the secretion of collagenase, elastase, and hyaluronidase. Inhibition of neutrophil function has been noted, and in vitro research demonstrates that curcumin inhibits 5-hydroxy-icosatetraenoic acid (5-HETE) in intact human neutrophils.72 Turmeric has been found to block cytokine-induced transcription of leukocyte adhesion molecules ICAM-1, VCAM-1, and E-selectin73, and it appears to induce the production of endogenous TGF-B1 in animal wounds.

**Antioxidant effects:** Turmeric has been reported to possess antioxidant properties in vitro and in animal studies.74-76 Turmeric preparations have been found to scavenge free radicals (peroxides) and phenolic oxidants, inhibit lipid peroxidation induced by chemical agents,77 and inhibit iron-dependent lipid peroxidation in rat tissues.78 In vitro research shows that turmeric may prevent oxidative damage to DNA79 and may be a potent scavenger of nitric acid.80 Curcumin appears to generate a hydroxyl radical.81
Anti-platelet aggregation effects: Curcumin inhibits thromboxane A2 without affecting the synthesis of prostaglandin I2.\textsuperscript{12} \textit{In vitro}, curcumin inhibits platelet aggregation induced by ADP, epinephrine, or collagen.\textsuperscript{83} Turmeric appears to inhibit arachidonic acid incorporation into platelet phospholipids, degradation of phospholipids, and cyclooxygenase.\textsuperscript{84}

Anti-proliferative effects: Multiple pre-clinical studies have explored potential anti-cancer mechanisms of curcumin.\textsuperscript{85-87} In a rat model, the effects of 0.2% or 0.6% dietary curcumin were evaluated on chemically induced colon adenocarcinoma.\textsuperscript{88} Histological examination after one year revealed both preventative and therapeutic benefits of curcumin when compared to animals not receiving curcumin, with better response at higher doses. Histologic examination revealed evidence of apoptosis of cancer cells. In mice, six weeks of a 2% curcumin diet was found to decrease cellular proliferation and increase apoptosis of implanted androgen-dependent LNCaP prostate cancer cells.\textsuperscript{89} However, \textit{in vitro} research on the anti-proliferative effects of curcumin on breast cancer cells reported no evidence of apoptosis.\textsuperscript{90} Dietary turmeric extract given to mice (2% or 5% of diet) significantly inhibited chemically-induced skin and gastric tumors. A 57% reduction in the incidence of chemically-induced colonic epithelial cell dysplasia was noted in mice fed a 2% curcumin diet.\textsuperscript{91}

Lipid-lowering effects: In rat models of hyperlipidemia, a diet of 0.5% curcumin for eight weeks significantly lowered serum low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total cholesterol, and triglyceride levels, possibly by enhancing the activity of hepatic cholesterol-7a-hydroxylase and increasing cholesterol catabolism.\textsuperscript{92} The turmeric constituents demethoxycurcumin, bisdemethoxycurcumin, and acetylcurcumin appear to inhibit \textit{-stimulated lipid peroxidation in rat tissues and liver microsomes}.\textsuperscript{78} In a rat model of hyperlipidemia, a 50% ethanolic extract of turmeric was associated with a significant reduction in the ratio of total cholesterol to phospholipids.\textsuperscript{93} In rabbits fed a high cholesterol diet, oral turmeric (1.6-3.2mg/kg) was associated with lower levels of plasma cholesterol and triglycerides than a control group, although no differences in atherogenesis were noted on histological examination of aortas.\textsuperscript{94}
Gastro-protective effects: Oral administration of turmeric to rats (500mg/kg) significantly reduces the incidence of chemically-induced duodenal ulcers and is associated with an increase in intestinal wall mucus and non-protein sulfhydryl content. However, early research in guinea pigs reported that various constituents of turmeric do not protect against histamine-induced gastric ulcerations.

Gallbladder effects: Gallbladder contraction over the two-hour period following the administration of 20mg curcumin has been demonstrated in humans. Animal research reports that curcumin in the diet reduces the incidence of chemically-induced gallstones in mice.

Hypoglycemic effects: Based on animal study, both curcuminoids and sesquiterpenoids in turmeric may exhibit hypoglycemic effects via PPAR-gamma activation.

Pharmacodynamics/Kinetics

Absorption: Animal research shows that the absorption of curcumin after oral administration varies from 25-60%, with most of the absorbed flavonoid being metabolized in the intestinal mucosa and liver. The remainder is excreted in the feces.

Distribution: Based on a clinical trial, Garcea et al. report that a daily dose of 3.6 g curcumin may achieve pharmacologically efficacious levels in the colorectum with negligible distribution of curcumin outside the gut.

Bioavailability: Authors of one clinical trial concluded that the lack of quantifiable curcumin in the plasma observed after a dose as high as 3,600 mg is consistent with recent clinical reports, in which oral doses of 30-180 mg curcumin failed to establish detectable plasma levels, and doses of 4,000-12,000 mg yielded curcumin peak levels of 0.5-2 mcM/L after one hour of administration. The authors also concluded that products of metabolic curcumin conjugation are present in the colorectum of humans who have ingested curcumin, but that these metabolites only contribute to a very minor extent.
of the overall colorectal load of curcumin-derived species. This finding is consistent with the idea that the pharmacologic effects of curcumin in the colorectum are probably caused by the parent compound and not by its metabolites.\textsuperscript{102}

**Pharmacodynamics:** In rats, curcumin is reported to be a potent inhibitor of cytochrome P450 (CYP) 1A1/1A2, a less potent inhibitor of CYP 2B1/2B2, and a weak inhibitor of CYP 2E1.\textsuperscript{102} Inhibition of cytochrome P450 has also been demonstrated \textit{in vitro}. Turmeric may decrease hepatocyte glutathione levels;\textsuperscript{103} curcumin appears to induce glutathione-S-transferase activity in mice.\textsuperscript{104}

Curcumin, a constituent of turmeric, completely inhibited mycelial growth of \textit{Aspergillus alliaceus} isolate 791 at 0.1\% (w/v) and decreased ochratoxin A production by approximately 70\% at 0.01\% (w/v).\textsuperscript{105}

In the checkerboard test, the ethyl acetate extract of \textit{Curcuma longa} L. markedly lowered the MICs of ampicillin and oxacillin against methicillin-resistant \textit{Staphylococcus aureus} (MRSA). In the bacterial invasion assay, MRSA intracellular invasion was significantly decreased in the presence of 0.125-2mg/mL of \textit{Curcuma longa} extract compared to the control group.\textsuperscript{106}

4. **Brain disorders**

A large population in India is suffering from brain disorders.\textsuperscript{107, 108} Anciently Epilepsy was considered as a supernatural event, and was thought as a form of demonic possession and was hard to treat with this belief even Depression which was called as melancholia and black magic was thought to be its causative agents. In 1906 Dr Alois Alzheimer, a German physician who had discovered Alzheimer’s disease after watching an elderly patient die of an unknown mental illness and later he proved it as a degenerative neurological disease. These diseases have been treated with herbal drugs by vaidha and hakeem from the olden times.
4.1 Depression and current therapy

The lifetime occurrence of this disorder is as high as 20% in the common population worldwide in ratio (male: female) 5:2. A large number of proportion become chronic and when follow up to 5 and 10 years found 12% and 7%, respectively, were still depressed. After recovery patients found high rate of recurrence, among them 75% of patients experienced major depression episode more than once within 10 years. Suicide is a considerable risk for mortality in depression, and the rate of suicide is rather high between the age of 15 and 24 years.

Pathophysiology of depression

Monoamine hypothesis

The catecholamine hypothesis of mood disorders was put forward in 1960's by Schildkraut. The deficiency of noradrenaline in certain brain circuits is associated with depression whilst an overabundance results in mania. These brain circuits are found in the brain stem, in the locus coeruleus, and project to many areas of the brain including the limbic system which is important in regulating emotions.

Evidence for noradrenaline depletion in depression also comes from the following observations:

i. Levels of metabolites of noradrenaline in urine and CSF are low in depressed individuals

ii. Increased densities of certain noradrenaline receptors in the cortex of depressed suicide victims.

iii. This pattern of 'up-regulation' occurs when the level of neurotransmitter in synapses is abnormally low and is a compensatory mechanism to pick up whatever signals are available.

iv. Drugs which selectively block noradrenaline reuptake such as reboxetine, increase nor-adrenaline in synapses and are effective antidepressants in many people.
Serotonin Hypothesis

Synaptic depletion of serotonin is implicated in depression. It may be that defects in neuronal circuits using serotonin could dampen noradrenaline signalling. Serotonin producing neurones project from the raphe nuclei in the brain stem to many regions of the brain including those that secrete noradrenaline. However, serotonin may also be more directly responsible for depression since there are serotonin producing neurones extending into areas of the brain thought to be involved in depressive symptoms - these include the amygdala which is involved in appetite, libido and sleep.

Evidence for serotonin depletion:

i. Tricyclic antidepressants produce many effects in the brain, including a decrease in serotonin reuptake and a consequent rise in serotonin levels in synapses.

ii. Selective serotonin reuptake inhibitors (SSRI's) such as prozac, block the reuptake of serotonin into the presynaptic neurone, they are hugely significant in the treatment of depression.

Endocrine processes in depression

A variety of hormonal abnormalities, such as altered levels of Cortisol, growth hormone (GH), or thyroid hormones, indicate the existence of endocrine disturbances, especially dysfunctions in the hypothalamic-hypophyseal-adrenal (HPA) axis and/or the regulation of thyroid function. The consistent finding that a significant subpopulation of depressed patients hypersecrete Cortisol during the depressed state but not after recovery led to intensive investigation and analysis of the HPA system. The observations include hypersecretion of hypothalamic corticotropin-releasing hormone (CRH) and inadequate glucocorticoid feedback, increased Cortisol levels, and impaired suppression of the HPA axis in response to exogenous glucocorticoid administration. A more refined analysis recently led to formulation of the concept that impaired corticosteroid receptor signaling is a key mechanism in the pathogenesis of depression.
Neuroimmune mediators

The immune system is a key mediator of brain-body interactions. Cytokines influence various CNS functions that are dysregulated in major depression, such as sleep, food intake, cognition, temperature, and neuroendocrine regulation. Experimental administration of interleukin-1 (IL-1) into the CNS produces stress-like effects on behavior, monoamine transmitters, HPA axis activity, and immune function; IL-1 is also a regulator of the 5-HT transporter gene. Another hint to the link between immune system and mood came from observations that a large number of previously psychiatrically healthy individuals treated with exogenous cytokines such as interleukin-2 (IL-2) and interferon-α (IFN-α) develop depression-like symptoms, such as depressed mood, increased somatic complaints, and stress reaction, cognitive impairment, and difficulties with motivation and flexible thinking. The fact that these are transient alterations, which disappear after termination of therapy, implies that cytokines may play a causal role in producing these symptoms.

Neurotrophins and depression

One hypothesis for the pathophysiology and treatment of depression involves adaptation or plasticity of neuronal systems. Depression could thus result from an inability to make the appropriate adaptive responses to stress or other aversive stimuli, and antidepressants may act by correcting this dysfunction or by directly inducing the appropriate adaptive responses. The neurotrophic factors are among the growth factors that have been studied for their role in the adult nervous system. Of these endogenous proteins, brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) have been shown to promote the function and growth of 5-HT-containing neurons in the adult brain. Chronic, but not acute, infusions of these substances have impressive effects on serotonergic neuron growth and regeneration, and further induced sprouting of 5-HT nerve terminals.

Sign and symptoms

According to the criteria of the Diagnostic and Statistical Manual of Mental Health, Fourth Edition (DSM-IV). Depression is a major disorder that affects a large number of...
population all over the world. It is not barred to age and can occur at any stage of life causes severe behavioral changes and distress in normal life. If left untreated, can be deadly. The main psychopathological symptoms involved are anhedonia, low or depressed mood, and low energy or fatigue. Other symptoms include feelings of guilt, sleep and psychomotor disturbances, suicidal tendencies, low self-esteem with autonomic and gastrointestinal distress. Depression is a heterogeneous disorder and a complex phenomenon of many subtypes and multiple etiologies. It concerned with progressive mood disturbances with or without psychotic features, and influence with other somatic and psychiatric disorders.

**Pharmacological treatment of depression**

The first-generation antidepressants, the tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs), increase the concentrations of 5-HT and/or NE and are effective in alleviating the symptoms of depression. Although both types of drugs have been used with great success for many years, there are several undesirable side effects that limit their application. TCAs acts on many other transmitter systems in the CNS and periphery, e.g., the histaminergic or acetylcholinergic systems, leading to sedation, hypotension, blurred vision, dry mouth, and other unwanted effects. In addition, TCAs may be life-threatening and fatal in overdose, especially due to their effects on the cardiovascular system. Also, the irreversible MAOIs have their own problems, such as an interaction with tyramine (the so-called “cheese effect”), which causes potentially lethal hypertension. The development of newer antidepressants has aimed to improve the safety and tolerability of the TCAs and reuptake inhibitors, and selectivity for a single monoamine seemed to be the key to this goal. Since the introduction of fluoxetine as the first selective serotonin reuptake inhibitor (SSRI), a great number of similarly acting drugs have followed and SSRIs are now applied in the treatment of several psychiatric disturbances, such as anxiety, panic, or obsessive compulsive disorder, where altered serotonergic transmission is assumed. Because preclinical and clinical studies have shown that chronic stimulation of the 5-HT system also affects the NE system and vice versa, there has been renewed interest in the role of neurotransmitters other than serotonin. The development of the newest generation of antidepressants, including reboxetine (a selective NE-reuptake inhibitor), venlafaxine (a dual reuptake inhibitor), or the multiple receptor-acting substances mirtazepine, nefazodone, bupropion, and
trazodone, may positively influence therapeutic potentials with reduced incidence of side effects due to reduced affinities for other systems. Interestingly, one drug, tianeptine, shows a quite atypical mechanism, namely an increase in 5-HT uptake, but most probably this substance predominantly counteracts stress effects in the hippocampus. 125

Table 3. 1 Chemical antidepressant Drugs approved by the FDA

<table>
<thead>
<tr>
<th>Generic</th>
<th>Drug Class</th>
<th>Starting Dose(mg/d)</th>
<th>Therapeutic Range(mg/d)</th>
<th>Half-Life (h)</th>
<th>Schedule</th>
</tr>
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<tbody>
<tr>
<td>Amitriptyline</td>
<td>TCA</td>
<td>25</td>
<td>150-300</td>
<td>12-24</td>
<td>qhs</td>
</tr>
<tr>
<td>Amoxapine</td>
<td>NSRI</td>
<td>100</td>
<td>200-300</td>
<td>8-30</td>
<td>Bid</td>
</tr>
<tr>
<td>Bupropion</td>
<td>NDRI</td>
<td>75</td>
<td>300-450</td>
<td>20</td>
<td>Tid</td>
</tr>
<tr>
<td>Citalopram</td>
<td>SSRI</td>
<td>10</td>
<td>20-40</td>
<td>35</td>
<td>Qd</td>
</tr>
<tr>
<td>Desipramine</td>
<td>TCA</td>
<td>25</td>
<td>150-300</td>
<td>21-23</td>
<td>Qd</td>
</tr>
<tr>
<td>Doxepin</td>
<td>TCA</td>
<td>25</td>
<td>150-300</td>
<td>17-51</td>
<td>qhs</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>NSRI</td>
<td>20</td>
<td>30-60</td>
<td>12</td>
<td>Bid</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>SSRI</td>
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<td>10-20</td>
<td>27-32</td>
<td>Qd</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>SSRI</td>
<td>10</td>
<td>20</td>
<td>4-16 d</td>
<td>Qd</td>
</tr>
<tr>
<td>Imipramine</td>
<td>TCA</td>
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<td>150-300</td>
<td>11-25</td>
<td>qhs</td>
</tr>
<tr>
<td>L-Deprenyl</td>
<td>MAOI</td>
<td>6</td>
<td>12</td>
<td>10</td>
<td>Qd</td>
</tr>
<tr>
<td>Maprotiline</td>
<td>NRI/TetraCA</td>
<td>25</td>
<td>150</td>
<td>43-90</td>
<td>qhs</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>Other</td>
<td>15</td>
<td>15-45</td>
<td>20-40</td>
<td>qhs</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>Other</td>
<td>200</td>
<td>600</td>
<td>2-4</td>
<td>bid-tid</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>TCA</td>
<td>10</td>
<td>75-150</td>
<td>16-44</td>
<td>qd</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>SSRI</td>
<td>10</td>
<td>20-40</td>
<td>24</td>
<td>qd</td>
</tr>
<tr>
<td>Phenelzine</td>
<td>MAOI</td>
<td>15</td>
<td>45-90</td>
<td>12</td>
<td>bid-tid</td>
</tr>
<tr>
<td>Protriptyline</td>
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<td>5</td>
<td>15-30</td>
<td>60-90</td>
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<tr>
<td>Sertraline</td>
<td>SSRI</td>
<td>50</td>
<td>50-200</td>
<td>26</td>
<td>qd</td>
</tr>
<tr>
<td>Tranylcypromine</td>
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<td>20-60</td>
<td>4-8</td>
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<tr>
<td>Trazodone</td>
<td>Other</td>
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<td>300-600</td>
<td>3-9</td>
<td>bid-tid</td>
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<tr>
<td>Trimipramine</td>
<td>TCA</td>
<td>25</td>
<td></td>
<td>11-23</td>
<td>qhs</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>NSRI</td>
<td>25</td>
<td>225-375</td>
<td>5-11</td>
<td>tid</td>
</tr>
</tbody>
</table>
Important Herbs used in Depression

**Lemon balm** (*Melissa officinalis*): quick-acting, gentle remedy that lifts the spirits. Melissa refers to the bees that so love this plant—we love it too for the sweetness it brings, especially for sadness accompanying mild depression and/or anxiety; particularly useful for menopausal depression; also a gentle digestive remedy; good tea, tincture, glycerite.

**Rosemary** (*Rosmarinus officinalis*): for depression with general debility, sluggish appetite, cardiovascular deficiency, and cloudy thinking (sometimes called "vegetative"); dispels despondency and worry; increases concentration and memory by increasing blood flow to brain; especially effective to dispel brain fog of menopause and aging; its warming effect can dispel chilly toes along with winter blues; great as tincture and added to soups.

**Lavender** (*Lavandula officinalis*): can be used for depression characterized by low motivation, energy, and appetite, as well as for depression coupled with anxiety; helpful when a sense of loss affects the heart; calms the mind while also stimulating gently; use in small proportions in tinctures or tea blends—the flavor is strong.

**Damiana** (*Turnera diffusa*): an excellent tonic in "vegetative" depression, as well as when depression is accompanied by anxiety; has a reputation for kindling sexual desire; a wonderful tea for winter-morning "blahs" or general feelings of mental or physical inertia.

**Saint John's wort** (*Hypericum perforatum*): while this plant has had some mixed press, it has proven itself in clinical settings and through research to be quite effective for mild to moderate unipolar depression and seasonal affective disorder; blooming in our fields right around summer solstice, it brings the sun's bright spirit into the depths of winter, elevating mood, and relieving fatigue and negative sense of self; best if combined with other mood-supporting herbs, especially lemon balm; not to be used with antidepressants or other prescription medications without professional advice; can take 4–6 weeks to reach full effectiveness; take as a tea, tincture, or capsule (standardized extract).
Valerian root (*Valeriana officinalis*)\(^1\): historically used for "feeble brain circulation" with depression and despondency; current research has found valerian to be as effective as a leading antidepressant (Elavil) when combined with St. John's wort; best as tincture.

Schisandra berry (*Schisandra chinensis*)\(^2\): invigorating to the mind as well as the digestion; excellent for exhausted and unfocused folks; also excellent for liver damage and regeneration (take with food—can give a little heartburn).

Eleuthero root (*Eleutherococcus senticosus*)\(^3\): a fairly "neutral" adaptogen, it's not too stimulating for most folks, but does increase energy and immune function in the short term and over time.

Holy basil leaf (*Ocimum sanctum*)\(^4\): like culinary basil, this herb supports digestion and enhances circulation, encouraging clear thinking; a great remedy to consider where blood sugar levels are high; a powerful anti-inflammatory and antioxidant; makes great tea or available as capsule or tincture.

4.2 Alzheimer's disease

Originally described by Alois Alzheimer in 1907, Alzheimer's disease (AD) has emerged as the most common type of dementia in the elderly today.\(^5\) Although the definitive diagnosis of AD requires histologic confirmation, in the absence of a readily discernible cause, the clinician may establish the diagnosis antemortem, with a fair degree of certainty, based on the clinical findings of a gradually progressive cognitive decline that results in the loss of memory, language skills, activities of daily living, and executive function.\(^6\)

Pathophysiology

The classic neuropathologic findings in AD include amyloid plaques, neurofibrillary tangles, and synaptic and neuronal cell death. Granulovacuolar degeneration in the hippocampus and amyloid deposition in blood vessels might also be seen on tissue examination.
Amyloid Plaques

Although amyloid plaques or senile plaques may be classified further according to their composition, all contain forms of β-amyloid protein (Aβ). Aβ is a 39- to 42-amino acid peptide that is formed by the proteolytic cleavage of β-amyloid precursor protein (APP) and is found in extracellular deposits throughout the central nervous system (CNS). Aβ is believed to interfere with neuronal activity because of its stimulatory effect on production of free radicals, resulting in oxidative stress and neuronal cell death.

Neurofibrillary Tangles

Neurofibrillary tangles are paired helical filaments composed of tau protein, which in normal cells is essential for axonal growth and development. However, when hyperphosphorylated, the tau protein forms tangles that are deposited within neurons located in the hippocampus and medial temporal lobe, the parietotemporal region, and the frontal association cortices, leading to cell death.

Neuron and Synapse Loss

Areas of neuronal cell death and synapse loss are found throughout a distribution pattern similar to that of the neurofibrillary tangles. The death of cholinergic neurons in the basalis nucleus of Meynert leads to a deficit in acetylcholine (Ach), a major transmitter believed to be involved with memory. In addition, loss of serotonergic neurons in the median raphe and adrenergic neurons in the locus ceruleus lead to deficits in serotonin and norepinephrine, respectively.

Chromosomal Mutations

Genetic mutations in chromosomes 21, 14, and 1 have been shown to cause familial early-onset AD. Inherited in an autosomal-dominant pattern, the chromosomal mutations account for less than 5% of all cases and result in the overproduction and deposition of Aβ. Chromosome 21, which codes for APP, was first evaluated for an association with AD when Down syndrome patients with the trisomy 21 aberration were observed to develop dementia in the fourth decade. Mutations in presenilin 1 (PS-1) on chromosome 14 and presenilin 2 (PS-2) on chromosome 1 also cause AD and are responsible for the majority of familial early-onset cases.
Inflammation

The exact role of inflammation in the pathogenesis of AD is still controversial. Although some studies have been able to demonstrate the presence of activated microglia (a marker of the brain's immune response) in patients with probable AD, a number of prospective clinical trials evaluating the use of drugs targeting various aspects of the immune system such as prednisone, hydroxychloroquine, and selective COX-2 inhibitors have been able to demonstrate only marginal benefits at best.144

Although some studies have suggested a neuroprotective role for nonsteroidal anti-inflammatory drugs, a recent large study of 351 patients revealed that these medications did not slow progression and cognitive decline in established mild-to-moderate Alzheimer's disease.145, 146

Signs and symptoms

AD is a progressive dementia with memory loss as the major clinical manifestation. Although short-term memory impairment is often the manifesting symptom, remote memory loss also appears to be affected over time. Another important feature of AD is the disturbance of language. Initially, AD patients might search for words when naming objects or while engaged in a simple conversation. But with progression of the disease, the language difficulties evolve into an inability to communicate as the patient struggles with a markedly limited vocabulary, nominal aphasia, and defects in verbal comprehension.

Other cortical signs and symptoms such as apraxia, acalculia, and visuospatial dysfunction may become apparent over the course of the disease. With the development of apraxia, patients lose the ability to carry out such simple tasks as combing their hair or turning on a water faucet. Acalculia may become evident when the patient is no longer able to maintain a checkbook or household accounts. Visuospatial abnormalities can be seen as patients become disoriented with their body position in space.

Behavioral problems emerge throughout the various stages of the disease. Mood disturbances such as depression, anxiety, or apathy may be present early on in AD,
whereas delusions, hallucinations, and psychosis can be prominent in later stages. In addition, aggression and inappropriate sexual behavior can be particularly problematic for the caregiver.

In advanced stages of AD, patients might exhibit extrapyramidal signs such as tremor and gait disturbance, frontal lobe release phenomena, urinary incontinence, and myoclonus. Seizures can also be seen in some patients with late-stage disease. Patients with end-stage AD almost invariably enter a vegetative state when all cognitive activity ceases.

**Treatment**

**Management of Cognition**

The major issues in treating AD are the improvement of memory and cognition and the delay of disease progression. At present there are no proven medications that cure or slow progression in Alzheimer's disease. Temporary improvements in cognition and behavior can be seen with the two existing drug classes of cholinesterase inhibitors and N-methyl-d-aspartate (NMDA) receptor antagonists.

The three cholinesterase inhibitors as well as the NMDA receptor antagonist memantine are listed in table 1. The new patch formulation of rivastigmine (Exelon) allows the convenience of once-daily administration, with a marked decrease in the common gastrointestinal (GI) side effects seen with the cholinesterase inhibitors. Efficacy appears to be similar among the cholinesterase inhibitors. The only reported differences are the dosing schedule and side-effect profile of each individual drug.

**Management of Noncognitive Symptoms**

Behavioral disturbances in AD may also be treated pharmacologically with both traditional and atypical neuroleptics. Although haloperidol can be effective, the atypical antipsychotics, which include risperidone, quetiapine, and olanzapine, may be better tolerated than traditional agents. There is not enough evidence to support the use of benzodiazepines, lithium, and anticonvulsants for the treatment of psychosis in patients with AD. Special care units within long-term care facilities are another consideration;
some studies have shown a reduced need for antipsychotics and physical restraints as well as a decrease in behavioral disturbances in AD patients who reside there.\textsuperscript{148} Psychosocial intervention for the caregiver is also an integral part of managing patients with AD. Education, support groups, and respite care services are should be suggested to family members.

**Table 3.2 Drug Therapy for Alzheimer's disease**

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetylcholinesterase Inhibitors</td>
<td>Donepezil, 5 mg once daily, can increase to 10 mg daily after 4-6 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rivastigmine tartrate, Pill: 1.5 mg bid initially, then titrate by 1.5 mg bid every 2 wk up to 12 mg daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patch: 4.6 and 9.5 mg patch size daily</td>
</tr>
<tr>
<td></td>
<td>Galantamine</td>
<td>4 mg bid initially, then titrate by 4 mg bid every 4 wk up to 24 mg daily</td>
</tr>
<tr>
<td>2</td>
<td>NMDA Receptor Antagonists</td>
<td>Memantine, 10 mg bid</td>
</tr>
</tbody>
</table>
Herbs used in the treatment of Alzheimer’s disease

*Curcuma longa*: Curcuma longa (Turmeric, Harida) has been used as a source of Curcumin (diferuloylmethane), an orange-yellow component of turmeric or curry powder. Studies have proved that Curcumin has anti-inflammatory and antioxidant activities, and it helps in combating Alzheimer's Disease (AD).63

*Bacopa monniera*: Goswami et al., evaluate the effect of Bacopa monnieri (Brahmi), associated with the Ayurveda system of medicine, on the cognitive functions in Alzheimer's disease patients, and conclude that it could be beneficial in these patients, but more study is needed.149

*Centella asiatica*: Extract from the leaves of Gotu Kola (Centella asiatica) has been used as an alternative medicine for memory improvement in the Indian Ayurvedic system of medicine for a long time.150

*Ginkgo biloba*: Ginkgo Biloba is the best known herb for Alzheimer's disease and its associated symptoms. In controlled clinical trials, using a placebo and control group, ginkgo biloba extracts showed therapeutic benefits in Alzheimer's, similar to prescription drugs such as Donepezil or Tacrin, with minimal undesirable side effects.151 The chief chemical constituent of gingko biloba is gingkolides and it is a pertinent antioxidant, with neuroprotective and cholinergic activities that help in the management of AD. Ginkgo biloba improves protection against Aβ protein-induced oxidative damages (degrading hydrogen peroxide, preventing lipids from oxidation, and trapping the reactive oxygen species).152

*Salvia officinalis*: Sage as it is more commonly referred for Alzheimer's disease treatment. It has been reported to assist the brain in the fight against AD. Sage contains the antioxidants carnosic acid and rosmarinic acid. These compounds are thought to protect the brain from oxidative damage.153
Rosmarinus officinalis: Rosemary contains the following natural COX-2 inhibitors: Apigenin, carvacrol, eugenol, oleanolic acid, thymol, and ursolic acid. If a synthetic COX-2 inhibitor could prevent Alzheimer’s disease, so could a natural COX-2 inhibitor. In addition, Rosemary contains nearly two dozen antioxidants and another dozen anti-inflammatory compounds. Some of the strongest antioxidant substances in the herb are carnosic acid and ferulic acid, which have even greater reported antioxidant activity than the widely common synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

Matricaria recutita: German Chamomile is said to stimulate the brain, dispel weariness, calm the nerves, counteract insomnia, aid in digestion, break up mucus in the throat and lungs, and aid the immune system. Chamomile can relieve anxiety, and in higher doses, leads to drowsiness, according to the University of Maryland Medical Center.

Melissa officinalis: Historically, Melissa officinalis (lemon balm) was believed to sharpen memory. Lemon balm can also temporarily improve cognitive decline as well as improve the mood for Alzheimer’s patients. Another study addressing the use of lemon balm for Alzheimer’s disease, concluded that Melissa officinalis is one of several plants that may be useful in the prevention and treatment of Alzheimer’s disease due to its ability to inhibit acetylcholinesterase and its antioxidant activity.

Glycyrrhiza glabra: Alzheimer’s disease is characterized by neuronal loss and the presence of extracellular senile plaques, whose major constituent is amyloid-β peptide (Aβ). In this study, we investigated the effects of a water extract of licorice (Yashtimadhu) on Aβ25-35-induced apoptosis in PC12 cells. Results suggest that GWE exerts a protective effect against apoptotic neuronal cell death induced by Aβ fragments. Extract from the licorice root is reported to treat or even prevent brain cell death in diseases like Alzheimer’s and its associated symptoms.

Galanthus nivalis: The chief chemical constituent of the Galanthus nivalis L. (common snowdrop) is Galanthamine, and this is an isoquinoline alkaloid. Acetylcholinesterase
(AChE) inhibitors, which are also called 'anticholinesterase drugs', have been recently approved as an promising treatment approach for AD. Galanthamine has been found to be the long-acting and specific inhibitor of the AChE enzyme and to potentiate cholinergic nicotinic neurotransmission by allosterically modulating the nicotinic acetylcholine receptors, which may be of additional value in the treatment of AD.\textsuperscript{159}

*Huperzia serrata*: Huperzia serrata is one of the genera in the Huperziaceae family (syn. Lycopodiaceae family). This genus, has been used for its memory-enhancing effect since ages in the Traditional Chinese Medicinal system (TCM), and is known to contain a large group of alkaloids called 'Lycopodium alkaloids'. Huperzine A, a novel Lycopodium alkaloid extracted from Huperzia serrata, is well known as a reversible, potent, and selective AChE inhibitor. It is also known as 'Qian Ceng Ta' in China, and Huperzine A has been used as a therapeutic agent for AD from centuries.\textsuperscript{160} As reported by researchers, taking Huperzine-A leads to a significant improvement in memory, concentration, and the learning capacity. Research has also shown that Huperzine-A substantially reduces the abnormally high radical activity both in the brains of elderly animals as well as in the blood of Alzheimer's patients. An experimental study in monkeys has shown that it reverses scopolamine-induced amnesia, suggesting that it may benefit the cognitive problems in Alzheimer's patients or those with other cognitive disorders.\textsuperscript{161}

*Commiphora whighitti* (Guggulu), a plant resin, contains the major constituent of guggulipid, which is guggulsterone. The guggulipid has been seen to be a potential cognitive enhancer for improvement of memory in scopolamine-induced memory deficits.\textsuperscript{162} Commiphora whighitti acts on impairment in learning and memory and decreased choline actyl transferase levels in hippocampus. However, Commiphora whighitti shows maximum effects on memory functions and the potential for dementia disorder.\textsuperscript{163}

*Lipidium Meyenii*: Lipidium Meyenii (maca), is known as Maca. Maca shows beneficial improvement in memory and learning. Black maca improves experimental memory
impairment, induced by ovariectomy, due in part, to its antioxidant and AChE inhibitory activities. Results demonstrated that black maca can enhance learning and memory in OVX (ovariectomized) mice and this effect might be related, at least in part, to its ability to reduce LPO (Lipid peroxidation) and AChE in OVX mice.\textsuperscript{164}

Panax Ginseng: Panax Ginseng (Ren-shen) contains saponins protopanaxadiol, protopantriol, and oleanolic acid saponins that are reported to have memory-enhancing action for the learning impairment induced by scopolamine.\textsuperscript{163} Ginseng grows in Northeastern Asia. The Ginseng root has been used in folk medicine in countries like China and Korea, for boosting Qi(energy), from ancient time. Ginseng has a history of medicinal use that goes back thousands of years. The ginseng extract has many uses, and claim to achieve and maintain both physical health and mental well-being.

Acorus calamus: Acorus Calamus possesses a beneficial memory enhancing property for memory impairment, learning performance, and behavior modification. Acorus Calamus inhibits the acetylcholinesterase (AChE). Acorus Calamus contains a majority of α- and β-asarone.\textsuperscript{165} In the Ayurveda medicine system, Acorous Calamus has been used for the treatment of memory loss and its related symptoms. Acorus Calamus also shows anti-inflammatory, antioxidant, antispasmodic, cardiovascular hypolipidemic, immunosuppressive, cytoprotective, antidiarrheal, antimicrobial, and anthelmintic activities.

Angelica archangelica: Angelica archangelica, also known as Dudhachoraa (Laghu Coraka), contains several substances that have the same kind of activity as drugs used for Alzheimer's disease. These substances do not cause the side effects observed with drugs, such as, nausea, stomach ache, insomnia, and so on. The same phytochemicals in Angelica archangelica can also increase blood flow in the brain. A study shows that chloromethane sub-fraction of a methanol extract inhibit AChE in-vitro.\textsuperscript{166}

Tinospora cordifolia: Tinospora Cordifolia (Guduchi) possesses a memory enhancing property for learning and memory in normal and memory-deficits animals. Tinospora
Cordifolia's mechanism for cognitive enhancement is by immunostimulation and synthesis of acetylcholine, this supplementation of choline enhances the cognitive function.  

*Magnolia officinalis*: The bark of Magnolia Officinalis (talauma) is used as a traditional memory enhancing agent in Chinese medicine for the treatment of neurosis, anxiety, stroke, and dementia. Magnolia Officinalis inhibits the memory impairment induced by scopolamine through the inhibition of AChE. The ethanolic extracts of M. officinalis, magnolol and honokiol, are reported to have antioxidant activity in vitro and in vivo.

*Bertholettia excels*: Although the name is Brazil Nuts, the most significant exporter of Brazil nuts is not Brazil, but Bolivia. In Brazil these nuts are called castanhas-do-Para. It has a high concentration of lecithin, which contains choline. Choline is a building block for acetylcholine. These building blocks enhance the concentration of acetylcholine in AD patients. Other plants that contain good amounts of lecithin are dandelion flowers, poppy seeds, soybeans, mung beans, horehound, ginseng, cowpeas, English peas, and lentils.

*Withania somnifera*: Active glycowithanolides of Withania somnifera (Ashawgandha) have a significant antioxidant function, which is accomplished by increasing the activities of superoxide dismutase, catalase, and glutathione peroxidase. Ashwagandha is also reported as a Nervine tonic that rejuvenates the cells and boosts energy.

### 4.3 Epilepsy

Seizures result from paroxysmal and excessive electrical neuronal discharges in the brain that cause a variety of clinical manifestations. The term *epilepsy* is usually restricted to those cases with a tendency for recurrent seizures. The identification of a seizure as a symptom and not a disease diagnosis is an important distinction. Seizures are the clinical manifestation of epilepsy; the challenge is to identify the disease that explains the symptom. Often the underlying disease is epilepsy, but at other times it may be a nonepileptic disorder that causes symptoms that resemble an epileptic seizure.
The term epilepsy encompasses a group of syndromes that vary in its associated pathology and seizure types. The diagnosis of the epileptic syndrome is one of the primary objectives undertaken when managing a patient with seizures.

**Pathophysiology**

Two sets of changes can determine the epileptogenic properties of neuronal tissues. Abnormal neuronal excitability is believed to occur as a result of disruption of the depolarization and repolarization mechanisms of the cell (this is termed the *excitability of neuronal tissue*). Aberrant neuronal networks that develop abnormal synchronization of a group of neurons can result in the development and propagation of an epileptic seizure (this is termed the *synchronization of neuronal tissue*).

A hyperexcitability of neurons that results in random firing of cells, by itself, may not lead to propagation of an epileptic seizure. Indeed, both normal and abnormal patterns of behavior require a certain degree of synchronization of firing in a population of neurons. Epileptic seizures originate in a setting of both altered excitability and altered synchronization of neurons. The excitability of individual neurons is affected by

- Cell membrane properties and the microenvironment of the neuron
- Intracellular processes
- Structural features of neuronal elements
- Interneuronal connections

The membrane properties and microenvironment of neurons, which maintain potential differences of electrical charge, are determined by selective ion permeability and ionic pumps. Excitatory neurotransmitters usually act by opening Na⁺ or Ca²⁺ channels, whereas inhibitory neurotransmitters usually open K⁺ or Cl⁻ channels. The mechanism of action of certain anticonvulsant medications is by Na⁺ or Ca²⁺ channel blockade, which likely prevents repetitive neuronal firing. Extracellular ionic concentrations also can contribute to neuronal excitability; for example, an increase in extracellular K⁺ concentrations (such as in rapid neuronal firing or dysfunction of glia, which are mainly responsible for K⁺ reuptake) causes membrane depolarization.
Various *intracellular processes* are controlled by genetic information. Neuronal excitability can be preprogrammed by DNA-controlled effects on cell structure, energy metabolism, receptor functions, transmitter release, and ionic channels. The mechanisms that induce these changes, either phasic or long term, appear to be linked to ionic currents, especially $\text{Ca}^{2+}$ influx. Intracellular $\text{Ca}^{2+}$ mediates changes in membrane proteins to initiate transmitter release and ion channel opening; it also activates enzymes to allow neurons to cover or uncover receptor sites that alter neuronal sensitivity. Various plastic or persistent changes in excitability can result by influencing the expression of genetic information through $\text{Ca}^{2+}$ influx. This may occur by selectively inducing genes to synthesize a protein for a specific reason. One example is the induction of the *c-fos* gene to produce *c-fos* protein in neurons involved in an epileptic seizure by the administration of pentylenetetrazol. The exact effects of this coupling are not known, but it provides a means to study the effects of neuronal excitation on cell growth and differentiation as a model for epilepsy, learning, and memory.\textsuperscript{172}

In regard to the *structural features of neuronal elements* in relation to epilepsy, the two primary regions of the brain that are involved in epilepsy are the cerebral neocortex and the hippocampus. In the neocortex, excitatory synapses are made primarily on the dendritic spines and shaft. The release of neurotransmitters at these sites gives rise to excitatory postsynaptic potentials. The inhibitory synapses are more prominent on the soma or proximal dendrites, and give rise to inhibitory postsynaptic potentials. The placement of these synapses effectively prevents distal excitatory events from reaching the axon hillock. Alterations of neuronal morphology, either spontaneously or as a response to injury, could enhance excitability with either an actual increase in the number of excitatory synapses or a decrease in the number of inhibitory synapses. Such alterations could consist of reduced dendritic branching with excitatory synapses placed closer to the axon hillock, or loss of spines, allowing more excitatory synapses to occur directly on the shaft. Lesions in the neuronal cell body or tracts lead to degeneration of the axon terminal and a new terminal may sprout to make contact with the vacated postsynaptic membrane. This may in turn lead to an increase in the excitatory potential of the neuron.\textsuperscript{173} $\text{Ca}^{2+}$ currents that occur predominantly at the dendrites causing a high-
amplitude prolonged depolarization that can evoke a rapid train of Na$^+$ action potentials (burst-firing of Na$^+$), which is followed by a prolonged after-hyperpolarization. These discharges are believed to contribute to the paroxysmal depolarization shifts and after-hyperpolarization in experimental epileptic foci.\textsuperscript{174}

Neurons are influenced by synaptic and nonsynaptic interconnections. Neurochemical transmission between neurons involves a number of steps that can be selectively altered to affect neuronal excitability. These steps result in the release of neurotransmitters into the synaptic cleft and the postsynaptic membrane, resulting in excitatory or inhibitory postsynaptic potentials via Ca$^{2+}$ and other second messengers. The transmitters are deactivated by enzymes, by reuptake into axon terminals, or by uptake by glia. The primary excitatory neurotransmitters in the central nervous system are the amino acids glutamate and aspartate. The primary inhibitory neurotransmitters in the central nervous system are gamma-aminobutyric acid (GABA) and glycine. Neurotransmitters and neuromodulators exert their effects by acting on receptors. Specific properties of receptors have been identified on the basis of the effects of certain agonist and antagonist agents, some of which are anticonvulsant drugs. GABA\textsubscript{A} receptor drugs, which activate Cl$^-$, appear to be more effective as anticonvulsants than GABA\textsubscript{B} receptor agents, which activate K$^+$. The GABA\textsubscript{A} receptor is of primary importance in absence epilepsy due to its role in the synchronization and desynchronization of thalamocortical pathways. The oscillatory and burst-firing of these circuits is attributed to neurons in the reticular nucleus of the thalamus and leads to synchronization and desynchronization of the electroencephalogram (EEG). Alterations of this mechanism produce absence seizures. Kainic acid, quisqualic acid, and N-methyl-d-aspartate (NMDA) are excitatory amino acid analogues used to define the classes of receptors responsive to glutamate and aspartate. NMDA antagonists are one potential mechanism for some of the anticonvulsants.

Two hypotheses are associated with cortical dysplasia, which is a frequent cause of medically intractable focal epilepsy. The first hypothesis suggests that epileptogenesis results from a change in the synaptic properties of interneurons. The second hypothesis suggests abnormal intrinsic properties in the neurons, such as a mutation in the ion channel.
Signs and symptoms

There have been many attempts at a classification system for epileptic seizures. The most widely used classification system is the one developed by the International League Against Epilepsy (ILAE), which is an electroclinical classification system (Table 2.3).\textsuperscript{175} This classification assumes that there is a one-to-one correlation between the phenomenology of the actual seizures and electrical abnormalities on the EEG seen with the seizure. This, however, is not always the case and these exceptions highlight the main weakness of the ILAE classification.

<table>
<thead>
<tr>
<th>Seizure Types</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial Seizures</td>
<td>EEG findings suggest focal onset</td>
</tr>
<tr>
<td>Simple Partial Seizures</td>
<td>Consciousness not impaired</td>
</tr>
<tr>
<td>Phonatory</td>
<td>Vocalization arrest of speech</td>
</tr>
<tr>
<td>With somatosensory or special sensory symptoms</td>
<td>Simple hallucinations</td>
</tr>
<tr>
<td>With autonomic symptoms or signs</td>
<td>Epigastric sensations, pallor, sweating, flushing, piloerection, pupillary dilation</td>
</tr>
<tr>
<td>With psychic symptoms</td>
<td>Disturbance of higher cortical function</td>
</tr>
<tr>
<td>Complex partial seizures</td>
<td>Consciousness impaired</td>
</tr>
<tr>
<td>Typical absence</td>
<td>Regular and symmetrical 3-Hz 3SWC on EEG</td>
</tr>
<tr>
<td>Atypical absence</td>
<td>Irregular slow SWC on EEG</td>
</tr>
<tr>
<td>Myoclonic Seizures</td>
<td>Polyspike or slow SWC on EEG</td>
</tr>
<tr>
<td>Clonic Seizures</td>
<td>Fast activity or slow SWC on EEG</td>
</tr>
<tr>
<td>Tonic Seizures</td>
<td>Low-voltage fast EEG</td>
</tr>
<tr>
<td>Tonic-Clonic Seizures</td>
<td>Rhythm of less than 10 Hz on EEG</td>
</tr>
<tr>
<td>Atonic Seizures</td>
<td>Poly SWC or low-voltage fast</td>
</tr>
</tbody>
</table>
Treatment

The first-line treatment of epilepsy is administration of an antiepileptic drug (AED). The selection of an appropriate AED is based on diagnosis of the epileptic syndrome of the patient (Table 2.4). First-line therapy for patients with focal seizures includes phenytoin, carbamazepine, and valproate. Drugs for adjunctive therapy for focal seizures include levetiracetam, topiramate, zonisamide, lamotrigine, gabapentin, oxcarbazepine, phenobarbital, and tiagabine. Drugs used in generalized seizures include valproate, lamotrigine, phenytoin, phenobarbital, and ethosuximide, which is specific for absence seizures.

Table 3.4 Characteristics of Various Antiepileptic Drugs (AEDs)

<table>
<thead>
<tr>
<th>AED</th>
<th>Efficacy</th>
<th>Dose</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Partial seizures, generalized seizures</td>
<td>200 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, rash, nausea, vomiting, hyponatremia</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Absence seizures</td>
<td>250 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, anorexia, nausea, vomiting, weight loss, diarrhea, abdominal pain, headache, mood changes, rash, hirsutism, and gingival hyperplasia</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>Partial seizures</td>
<td>300 mg/day at 24-hr intervals</td>
<td>Neurocognitive effects, weight gain, mood changes, dry mouth, periorbital edema, myalgias</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>Partial seizures</td>
<td>100 mg/day at 1-wk intervals</td>
<td>Neurocognitive, nausea, vomiting, cardiac conduction abnormalities</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Generalized and partial seizures</td>
<td>50 mg/day at 2-wk intervals</td>
<td>Neurocognitive effects, headache, rash, mood changes, nausea, vomiting</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>Partial seizures</td>
<td>1000 mg/day at 2-wk intervals</td>
<td>Neurocognitive effects, mood changes, behavior changes, anesthesia</td>
</tr>
<tr>
<td>Medication</td>
<td>Seizure Type</td>
<td>Dosage</td>
<td>Adverse Effects</td>
</tr>
<tr>
<td>-------------</td>
<td>------------------------</td>
<td>-------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>Partial seizures</td>
<td>600 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, rash, nausea, vomiting, hyponatremia</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Generalized and partial seizures</td>
<td>30 mg/day at 4-wk intervals</td>
<td>Neurocognitive effects, mood changes, nausea, vomiting, rash, porphyria exacerbation, physical dependence</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Generalized and partial seizures</td>
<td>100 mg/day at 4-wk intervals</td>
<td>Neurocognitive effects, hirsutism, gingival hyperplasia, nausea, vomiting, coarse facies, headache, lymphadenopathy, osteomalacia</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>Partial seizures</td>
<td>150 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, weight gain, peripheral edema</td>
</tr>
<tr>
<td>Tiagabine</td>
<td>Partial seizures</td>
<td>4-8 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, mood changes, asthenia, nausea, vomiting</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Generalized and partial seizures</td>
<td>25-50 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, language problems, psychomotor slowing, mood changes, paresthesia, weight loss, renal stones</td>
</tr>
<tr>
<td>Valproate</td>
<td>Generalized and partial seizures</td>
<td>250 mg/day at 1-wk intervals</td>
<td>Neurocognitive effects, weight gain, nausea, vomiting, headache, hair loss, menstrual irregularities</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>Partial seizures</td>
<td>100 mg/day at 2-wk intervals</td>
<td>Neurocognitive effects, mood changes, insomnia</td>
</tr>
</tbody>
</table>

In patients who do not respond to medication, epilepsy surgery is a potential mode of treatment that can offer up to a 70% to 90% chance of seizure freedom (defined as no seizures or auras only, on medication) in some patients. Other novel modes of therapy include the vagal nerve stimulator (VNS), which is usually reserved for those patients with intractable epilepsy who are not surgical candidates. The VNS usually is as effective as a typical AED and usually does not provide a seizure-free state. Its mechanism of
action is not known. The benefits of the VNS as opposed to AEDs is that it does not have the neurotoxicities associated with AEDs. Some adverse effects of the VNS include coughing, hoarse voice, bradycardia, and exacerbation of sleep apnea. Neurocognitive side effects include dizziness, drowsiness, unsteadiness, blurred vision, ataxia, tremor, nystagmus, impaired memory, and fatigue.

**Important herbs used in the treatment of Epilepsy**

**Valerian Root** *Valeriana officinalis*: Valerian is currently one of the most popular orthodox antispasmodic medications in Russia and Germany according to Daniel Mowrey author of *Herbal Tonic Therapies*. It is its anticonvulsant action that has been useful in treating epilepsy. Valerian was used in the First World War to prevent shell shock in front-line troops. It can regulate and balance opposite extremes. Recent research has shown it to be a sedative but more research has reported it can also stimulate in a way as to improve coordination, increase concentration and energy. This tonic nature of Valerian allows it to depress or stimulate where necessary depending on the current needs of the nervous system. Another way Valerian has been characterized by clinical studies is that it has neurotropic effects directly on higher centers of the central nervous system. One of the most remarkable aspects of Valerian is the almost total lack of toxicity, even with long term use.

**Lobelia Herb** *Lobelia inflata*: Clinical studies have proven the antispasm action of Lobelia. Historically it has been used to treat epilepsy.

**Ginseng Root (Chinese)** *Panax ginseng*: Chinese Ginseng, perhaps the most famous medicinal plant of China, is considered as a tonic to whole body and has folk use for this condition.

**Mistletoe Herb** *Viscum album*: Mistletoe has a historical use for epilepsy but no recent studies that I know of have focused on this condition. Hippocrates claimed it was highly effective remedy for the spleen and some modern European physicians believe treating
the spleen may be beneficial in epilepsy. There has been confusion about the toxicity of this herb but paying attention to the correct botanical and current safety warnings, the herb can safely be used.\textsuperscript{180}

**Motherwort Herb** *Leonurus cardiaca*: Motherwort was used to calm epileptics during the 17th century and now is used as a nerve tonic and sedative. Current evidence has confirmed its benefits as a cardiotonic and hot-water extracts also show sedative and ant-epileptic effects in animals.\textsuperscript{181}

**Mugwort Herb** *Artemesia vulgaris*: Extracts of Mugwort have been injected into laboratory animals confirming its sedative effects so researchers conclude it is possible the herb could be beneficial for epilepsy. Mugwort has been used for this condition.\textsuperscript{182}

**Sage Leaf** *Salvia officinalis*: Sage is famous throughout history in many different cultures as a miracle herb. A constituent in a Chinese variety *Salvia miltiorrhiza* may become the source of a new tranquilizing agent but without the side-effects of Valium. Valium and Librium are benzodiazepines which are widely prescribed since 1960 to treat epilepsy. Benzodiazepines act on the central B\(_2\) receptors in the central nervous system. The herb compound also interacts with the central B\(_2\) receptors.\textsuperscript{183}

**Scullcap Herb** *Scutellaria lateriflora*: Scullcap has always been known as a mild and safe nervine. Traditionally it has been used for delirium tremens, St. Vitus’ dance, convulsions, seizures, hysterical states, lockjaw, tremors and epilepsy.\textsuperscript{184}

**Blue Vervain Herb** *Verbena hastate*: Blue Vervain is worth mentioning here after reading old American herb doctors tales of their successes with stubborn cases of epilepsy. Blue Vervain is another wonderful herb nervine use by many cultures all over the world. It is an American Indian remedy for several diseases including nervous afflictions.\textsuperscript{185}
Black Cohosh Root *Cimicifuga racemosa*: Black Cohosh is so highly recommended in numerous respected publications. Like many of the herbs it is considered as a sedative and antispasmodic and has been extensively used for epilepsy.\(^{186}\)

5. **Free radicals and their involvement in the selected brain diseases**

Free radicals are generated in all chemical and biological systems. In a living organism, the levels of free radicals generation are controlled by an antioxidant defence cascade, which prevent the oxidative damage to biomolecules like protein, lipid, DNA etc. In humans, most of the neurological diseases are the consequences of ‘oxidant-antioxidant’ imbalance which led to increased oxidative damage.\(^{187,188}\)

5.1 **Concept of free radical and its generation**

In a structure of atom, electrons are associated in pairs, each of them are found moving within a definite space called orbitals. Each electron pair has a spin quantum number +1/2 and -1/2. Free radicals are having independent existence containing unpaired electron and an unpaired electron found alone in its orbital. The simplest free radical is an atom of the element hydrogen, with one proton and a single electron. Examples of oxygen free radicals found in living system (i.e. with the unpaired electron located on O) are superoxide (\(O_2^-\)) and hydroxyl (\(\text{OH}^+\)), and nitric oxide (\(\text{NO}^+\)). Radicals add together like atomic hydrogen forms diatomic hydrogen (eqn [I]), and superoxide reacts with nitric oxide (eqn [II]). In all cases, a nonradical is formed. This is usually less reactive than the parent radicals (e.g. \(\text{H}_2\) is less chemically reactive than H), but not always. For example \(\text{ONOO}_2\) is more damaging to human tissues than either \(O_2^-\) or \(\text{NO}^+\). Most biological molecules are nonradicals.\(^{189}\)

\[
\begin{align*}
\text{H}^+ + \text{H}^+ & \rightarrow \text{H}_2 & & \text{[I]} \\
\text{O}_2^- + \text{NO}^+ & \rightarrow \text{ONOO}^- & & \text{(peroxynitrite)} & & \text{[II]}
\end{align*}
\]
When a free radical reacts with a nonradical, a new free radical is generated. For example, OH• reacts with hydrocarbons (including the fatty acid side-chains of membrane lipids) to abstract H. and leave behind a carbon-centred radical (eqn [III]).

\[
\text{\[
\text{CH} + \text{OH}^\bullet \rightarrow \text{C}^\bullet + \text{H}_2\text{O}\]
\]}

This process can start the free radical chain reaction of lipid peroxidation. A reactive free radical such as OH• abstracts hydrogen from a fatty acid side-chain as above. The resulting carbon-centred radicals react with oxygen (eqn [IV]).

\[
\text{\[
\text{C}^\bullet + \text{O}_2 \rightarrow \text{C}^\bullet - \text{O}^\bullet - \text{O}^\bullet \text{peroxyl radical}
\]}

Peroxyl radicals attack membrane proteins, and can also attack adjacent fatty acid side-chains (eqn [V]). The C• radical reacts with O₂ to give another peroxyl radical and the chain reaction continues. Hence, attack of a single OH• can cause oxidation of multiple fatty acid sidechains to lipid hydroperoxides. The polyunsaturated fatty acid side-chains essential to the fluidity of membranes are the most susceptible to free radical attack and subsequent lipid peroxidation.¹⁸⁹

\[
\text{\[
\text{C}^\bullet - \text{O}^\bullet - \text{O}^\bullet + \text{CH} \rightarrow \text{C}^\bullet - \text{O}^\bullet - \text{OH} + \text{C}^\bullet \text{lipid hydroperoxide}
\]}

As a different example, OH• adds to the purine base guanine in DNA, but again a new radical is generated, an 8-hydroxyguanine radical (eqn [VI]).

\[
\text{\[
\text{Guanine} + \text{OH}^\bullet \rightarrow [8\text{-hydroxyguanine}]^\bullet \text{[VI]}
\]}

This can undergo oxidation (loss of one electron) to the mutagenic lesion 8-hydroxyguaninone, which can lead to GCTA transversions.¹⁹⁰
5.2 Oxidative stress

The term oxidative stress refers to the situation of serious imbalance between production of reactive species and antioxidant defence. In principle, oxidative stress can result from:

1. Diminished antioxidants, e.g. mutations affecting antioxidant defense enzymes (such as CuZnSOD, MnSOD and GSHPX) or toxic agents that deplete such defences. For example, many xenobiotics are metabolized by conjugation with GSH; high doses can deplete GSH and cause oxidative stress even if the xenobiotic is not itself a generator of reactive species. Depletions of dietary antioxidants and other essential dietary constituents can also lead to oxidative stress, e.g. in kwashiorkor.

2. Increased production of reactive species, e.g. by exposure to elevated levels of toxins that are themselves reactive species (e.g. nitrogen dioxide gas, NO$_2^-$) or are metabolized to generate such species, or by excessive activation of ‘natural’ reactive species-producing systems (e.g. inappropriate activation of phagocytic cells in chronic inflammatory diseases, such as rheumatoid arthritis and ulcerative colitis). Mechanism 2 is usually thought to be more relevant to human diseases and is frequently the target of attempted therapeutic intervention, but the antioxidant nutritional status of sick patients may often be compromised.
5.3 Consequences of oxidative stress

Oxidative stress can result in the following.

1. Adaptation: e.g. by upregulation of antioxidant defence systems. For example, if adult rats are gradually acclimatized to elevated O\textsubscript{2}, they can tolerate pure O\textsubscript{2} for much longer than control rats, apparently owing to increased synthesis of antioxidant defence enzymes and of GSH in the lung. Ischaemic preconditioning provides another example. A brief period of ischaemia leads to depression of contractile function, and administration of antioxidants to the animals offers protection. However, repeated periods of ischaemia lead to quicker return of contractile function on reperfusion, but this adaptive response is blocked in the antioxidant treated animals. Hence ROS produced by ischaemia are initially damaging but also lead to a response protective against subsequent insult.

2. Tissue injury: oxidative stress can cause damage to all molecular targets; DNA, proteins and lipids (lipid peroxidation). Often, it is not clear which is the first point of attack, since injury mechanisms overlap widely. Indeed, the primary cellular target of oxidative stress can vary depending on the tissue under study. For example, when H\textsubscript{2}O\textsubscript{2} is added to many mammalian cells, increased DNA strand breakage occurs before
detectable lipid peroxidation or oxidative protein damage. This DNA damage appears to involve conversion of $\text{H}_2\text{O}_2$ into $\text{OH}^\cdot$ in the cell nucleus, by reaction with transition metal ions. Oxidative stress can halt cellular proliferation and in some cases induce senescence. Ironically, low level oxidative stress stimulates proliferation in some cell types.

3. **Cell death:** this can occur by essentially two mechanisms, necrosis, and apoptosis. Both can result from oxidative stress. In necrotic cell death, the cell swells and ruptures, releasing its contents into the surrounding area and affecting adjacent cells. Contents can include antioxidants such as catalase or GSH, and pro-oxidants such as copper and iron ions. Hence even if a cell dies by mechanisms other than oxidative stress, necrotic cell death can lead to oxidative stress in the surrounding environment. In apoptosis, the cell’s own intrinsic ‘suicide mechanism’ is activated; apoptosing cells do not release their contents and so apoptosis does not, in general, cause damage to surrounding cells. Apoptotic cell death may be accelerated in certain diseases, such as some of the neurodegenerative diseases, and oxidative stress has been implicated.

5.4 **Reactive Species and Human Disease**

Free radicals and other reactive species have been implicated in the pathology of over 100 human diseases, ranging from ulcerative colitis and hemorrhagic shock to cystic fibrosis and AIDS. Some human diseases may be caused by oxidative stress. For example, ionizing radiation generates $\text{OH}^\cdot$ by splitting water molecules and many of the biological consequences of excess radiation exposure are probably due to oxidative damage to proteins DNA and lipids. The symptoms produced by chronic dietary deficiencies of selenium (e.g. Keshan disease) or of tocopherols (neurological disorders seen in patients with defects in intestinal fat absorption) may also be mediated by oxidative stress since selenium is an essential cofactor for GSHPX enzymes and thioredoxin reductase and vitamin E is an important protector against lipid peroxidation. Persistent damage to DNA by reactive species may play a role in the initiation of some human cancers by creating mutagenic lesions such as 8-hydroxyguanine. However, reactive species are the primary cause of the condition in few diseases. More often, their increased formation is a consequence of the disease pathology, for the reasons summarized in Figure1. In many cases, the resulting oxidative stress makes a significant further contribution to tissue injury. For example, injury to vascular endothelium by turbulent blood flow, viral infections, or circulating toxins can initiate the process of

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atherosclerosis. Recruitment of monocytes to the injured vessel wall and their development into macrophages, followed by the free radical-mediated peroxidation of low-density lipoproteins within the vessel wall, play a key role in the development of atherosclerotic lesions. Antioxidant inhibitors of lipid peroxidation, such as the drug probucol, have a significant anti-atherosclerotic effect in animals and humans, and low dietary intake of vitamin E is a risk factor for the development of cardiovascular disease. Other diseases in which oxidative stress may play an important role in tissue injury include rheumatoid arthritis, inflammatory bowel disease, acute respiratory distress syndrome (ARDS), emphysema, and cancers related to chronic inflammation. There is growing evidence that oxidative stress is especially important in the pathology of Alzheimer’s disease.192

5.5 Consequences of oxidative stress in human disease192

Although oxidative stress probably occurs in all diseases, it is not necessarily important in the disease pathology. It may be a late stage in tissue injury, accompanying cell death rather than causing it. It may lead to sufficient induction of antioxidant defence mechanisms to protect the tissue. The following criteria should be met before concluding that reactive species (RS) are important in a given disease.

1. It should be possible to demonstrate RS presence at the site of injury, either directly and/or by measuring specific, validated ‘biomarkers’ of oxidative damage to biomolecules.

2. The time course of RS formation and/or of oxidative damage should precede or parallel that of tissue injury.

3. Direct application of RS or their generation within the tissue should reproduce most or all of the tissue damage observed in the disease.

4. Scavengers of RS, or other antioxidants, should decrease tissue injury to an extent related to their scavenging action or ability to prevent oxidative damage (as indicated by the levels of appropriate biomarkers). The development of novel therapeutic antioxidants by several companies is under way, especially for the treatment of cardiovascular and neurodegenerative disorders.
Table 3.5 Macromolecules targeted by oxidative stress

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Target of damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Low levels of oxidative base damage products are present in DNA isolated from all aerobic cells; levels often increase in patients with chronic inflammatory diseases or subjected to oxidative stress, e.g. from smoking. Some base damage products are excreted in urine, presumably resulting from DNA repair processes. Smokers and rheumatoid arthritis patients excrete more 8-hydroxydeoxyguanosine (8-OHdG). Elevated 8-OHdG concentrations are frequently observed in animals treated with carcinogens or other toxins.</td>
</tr>
<tr>
<td>Protein</td>
<td>Attack of ROS upon proteins produces carbonyls and other amino acid modifications (e.g. methionine sulfoxide, valine hydroxides, 2-oxohistidine, protein peroxides, hydroxylation of tyrosine to dopa, conversion of tryptophan to formylkynurenine). Low levels of carbonyls and certain other products (e.g. ortho-tyrosine, valine oxidation products) have been detected in healthy animal tissues and body fluids. Nitrotyrosines, products of attack on tyrosine by RNS, have been detected in atherosclerotic lesions, human plasma and urine; concentrations are higher in body fluids/tissues from patients with chronic inflammatory diseases. Bityrosine has been detected in urine and atherosclerotic lesions. Oxidized proteins are degraded by the cellular proteasome system: impairment of this system may cause accumulation of oxidized and nitrated protein aggregates in the major neurodegenerative diseases.</td>
</tr>
<tr>
<td>Lipid</td>
<td>Accumulation of ‘age pigments’ in tissues. Lipid peroxidation in atherosclerotic lesions. Presence of specific end products of peroxidation (e.g. isoprostanes) in body fluids (including urine); levels increase in plasma during oxidative stress, e.g. in smokers, in CCl4 treatment of animals, and in premature babies.</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Attacked by several ROS to generate allantoin, cyanuric acid, parabanic acid, oxonic acid and other products, which are present in human body fluids. Levels of these products increase in chronic inflammatory/metal-overload diseases.</td>
</tr>
</tbody>
</table>

5.6 Role of oxidative stress in epileptic seizures
Seizure generation may be related to the homeostatic imbalance of antioxidants and oxidants. To date, various experimental seizure models have been developed to investigate the role of endogenous antioxidants in response to excitotoxic oxidative stress. Impairment of endogenous antioxidant factors against oxidative stress is involved in seizure generation. Antiepileptic drugs, at least in part, impair antioxidant systems. The ability of antioxidants to attenuate seizure generation and the accompanying changes in oxidative burden further support an important role of antioxidants as having putative antiepileptic potential. Superoxide radicals are highly reactive and can initiate pathological oxidative metabolism, leading to the oxidation of macromolecules. The nervous system contains antioxidant enzymes, including SOD and GPx, that are expressed in higher quantities than CAT.\(^{193}\)

This spectrum of enzymatic defenses suggests that the brain may efficiently metabolize superoxide, but may have difficulty in eliminating the hydrogen peroxide produced by this reaction (i.e., superoxide dismutation). Hydrogen peroxide accumulation is of major concern, as the brain contains large quantities of iron and copper, which may catalyze the formation of hydroxyl radicals that can induce lipid peroxidation.\(^{194}\) Enhanced hydrogen peroxide, in turn, is reduced to water by peroxidases, mostly GPx (and Prx) in the brain. GPx levels in neuronal tissue appear to be relatively low for the prevention of peroxide insults. Furthermore, the neuronal cell membrane contains high levels of polyunsaturated fatty acids.\(^{195}\) Thus, brain cells may be extremely susceptible to peroxidative damage, reflecting the critical role of homeostasis with respect to antioxidant vs. peroxidative stress in the epileptogenesis.

**Mitochondrial dysfunction**

The brain is particularly susceptible to oxidative damage due to its high aerobic metabolic demand and high iron load.\(^{188}\) The brain is rich in mitochondria, the principal source of cellular superoxide \(O_2\cdot^-\) formed during respiration.\(^{196}\) It is plausible that prolonged seizures result in sufficient \(O_2\cdot^-\) production to overwhelm the endogenous mitochondrial antioxidant defenses by a cascade of events initiated by increased neuronal firing, excessive glutamate release, N-methyl-D-aspartate (NMDA) receptor activation, cytosolic and mitochondrial calcium influx, and increased ATP consumption.
Mitochondrial dysfunction has been reported to play a role in epilepsy in humans and in several animal models of epileptic seizures. The role of mitochondrial dysfunction arising from mitochondrial DNA mutation/depletion has been shown to be the cause of certain types of epilepsy. Epileptic seizures are a common phenotype of the inherited mitochondrial disease myoclonic epilepsy with ragged-red fibers, the first epilepsy in which a molecular defect was identified and linked with the epilepsy syndrome. One possibility is that mitochondrial free radical generation and resultant dysfunction may contribute to the epileptic seizures associated with mitochondrial diseases.

Growing evidence suggests that defects in oxidative phosphorylation complexes can result in increased $\text{O}_2^-$ production. Seizure activity can be induced by paradigms that increase the mitochondrial free radical load, such as local infusion of reductive iron salts or mitochondrial toxins and age-related neuronal disorders. Further, synaptic NMDA receptor activation, which results in increased $\text{O}_2^-$ production, is a necessary factor for seizure generation. For example, overproduction of mitochondrial $\text{O}_2^-$ resulted in seizure activity in Mn-SOD mice. Mn-SOD mice have a severe mitochondrial disease and behavioral manifestations including seizures. Seizure severity in Mn-SOD mice correlated with mitochondrial aconitase inactivation with advancing age.

**Pentylenetetrazol (PTZ)-induced seizures**

PTZ is a selective blocker of the GABA-A receptor chloride ionophore complex. It has convulsant effects after repeated or single administration and it affects several neurotransmitter systems, such as GABAergic, adenosinergic, and glutamatergic systems. After PTZ-induced seizures, significant decreases in GSH, GSSG, cysteine, and protein thiols as well as increases in the protein carbonyl and protein disulfides levels were observed in the mouse cerebral cortex. Previous work in the mouse hippocampus showed that protein thiol levels were decreased without significant alterations of GSH and GSSG, while protein disulfides, MDA equivalent, and carbonyl levels were increased in the time period shortly after a single treatment with PTZ. A previous demonstration showed that a single convulsive dose of PTZ resulted in significant changes in many parameters such as GABA-A receptor density and function, whole brain hydroxyl
Oxygen radicals damage found in AD includes advanced glycation end products, nitration, lipid peroxidation adduction products as well as carbonyl-modified neurofilament protein and free carbonyls. The cytopathological significance of oxidative damage is seen by the up-regulation of antioxidant enzymes. In later studies, we demonstrated that increased HO-1 expression co-localizes with the altered form of tau (τ). It has been found that while stable glycation products are predominantly associated with NFT and amyloid-β (Aβ) deposits, reversible or rapidly degraded adduction products are primarily in the cytoplasm of vulnerable neurons. This data means that oxidative damage is not restricted to long-lived lesions. In fact, when damage to short-lived molecules is examined, the damage is restricted to cytosolic compartments with NFT and Aβ being inversely correlated.

Proteins fail in the latter aspect of these criteria because cross-link modifications slow their turnover. Therefore, modifications associated with cross-linking of proteins, while useful to assess history, may reveal less of the current state. However, 8-hydroxyguanosine (8-OHG), a nucleic acid modification predominantly derived from hydroxide (•OH) attack of guanidine, is amplified in cytoplasmic RNA in vulnerable neuronal populations. Notably, cases of AD with the most extensive Aβ deposits show the lowest 8-OHG levels. These findings seem markedly contradictory if we consider Aβ to be the toxic agent in AD, which is likewise proposed to be a major source of oxidative free radicals. However, neurons containing NFT also have extremely low levels of 8-OHG (ie, current oxidative stress status) despite an obvious history of oxidative damage (ie, advanced glycation endproducts or lipid peroxidation). This suggests that both Aβ and NFT may serve an antioxidant function, and thus be cellular compensations for increased oxidative stress. The hypothesis that Aβ plays an antioxidant function is supported by a study of Down syndrome patients, a disease where Aβ deposits begin in the late teens and in which oxidative stress has been implicated.
Mitochondrial Dysfunction

Mitochondria are a potential major source of oxidative radicals and oxidative precursors, in the form of $O_2^-\text{ and H}_2\text{O}_2$, respectively, since their production is linked to metabolism. In early studies, increases in mitochondrial DNA in the cell of AD susceptible neurons, which in itself might cause increased oxidative potential. Perhaps more importantly, in situ hybridization studies with a chimeric cDNA probe to a common mitochondrial mutation (5 kilobase (kb) common deletion) have shown at least a 3-fold increase in AD cases compared to controls. Ultrastructural localization of mtDNA with colloidal gold shows that deleted mtDNA is mainly found in abnormal mitochondria (ie, those lacking cristae, swollen and in many cases fused with lipofuscin). These findings suggest that the mtDNA in situ hybridization detected mtDNA proliferation, deletion, and duplication in abnormal mitochondria, many of which have been fused with lysosomes, indicating that they are being turned over.

Quantitative analysis of the colocalization of the mtDNA deletion and 8-OHG in AD cases demonstrate a strong positive correlation ($r = 0.934$). However, mitochondrial DNA, even that containing the 5 kb deletion, is relatively spared from oxidative damage, like the formation of 8OHG, in comparison to cytoplasmic nucleic acid (ie, RNA). It is suspected that mitochondrial abnormalities correlate with, but do not directly cause, reactive oxygen species (ROS). This may be due to the fact that hydroxide radicals, which are responsible for the formation of 8-OHG, have a sphere of diffusion of only 2 nm and are fairly short lived. Therefore, since damage is topographically distinct, it is likely that OH radical formation occurs in the cytoplasm rather than the mitochondria and that they are unable to diffuse through the mitochondrial membrane to affect mtDNA. However, abnormal mitochondria may produce excess H$_2$O$_2$ through the conversion of O$_2^-\text{ by mitochondrial superoxide dismutase (SOD). Such H}_2\text{O}_2$ is readily diffusible and relatively stable, that is, until interacting with redox-active transition metals where the Fenton reaction produces hydroxyl radicals.
Several studies have implicated imbalances of trace elements, including Al, Si, Pb, Hg, Zn, Cu, and Fe in AD. A disruption in the homeostasis of the latter two redox-active metals is particularly significant in light of the increases in oxidative stress parameters, such as lipid peroxidation, and the oxidative damage to NFTs, senile plaques and nucleic acids.214, 221

Micro particle-induced X-ray emission, recently found that Zn(II), Fe(III), and Cu(II) are significantly increased in AD neuropil and that these metals are more concentrated within the core and periphery of senile plaques.222 These results support earlier studies reporting increased levels of iron, transferrin, and ferritin in AD. An in situ iron detection method revealed a significant association of redox-active iron with both NFT and senile plaques (SP) in AD. The association of iron with NFT may be partly related to the binding of iron to τ, their primary protein constituent.223

A hypothesis implicating free radicals could explain both the heterogeneous presentation of AD and the fact that aging is a significant risk factor for its development. Three key facts support this hypothesis224:

1. Neurons are extremely sensitive to free radical attack because their glutathione content is very low, their membranes are high in PUFAs, and brain metabolism requires a substantial amount of oxygen.
2. AD brain lesions are associated with typical free radical damage, e.g. DNA damage, oxidized protein, oxidized lipids, and glycosylated end products,
   i. Oxidized DNA is observed in the cerebral cortex of AD patients.225
   ii. Many studies have shown increased lipid peroxidation in the AD brain.
   iii. Protein oxidation is more marked in AD patients in the regions showing the most pathophysiologic changes.226
   iv. Several studies have identified elevated concentrations of oxidation end products in AD brains, including malondialdehyde, peroxynitrite, carbonyls,
advanced glycosylated end products (AGEs), and various enzymes associated with oxidative stress.\textsuperscript{227}

3. Metals capable of catalyzing free radical production are naturally present in the brain, e.g. iron, copper, zinc, and aluminum.\textsuperscript{222}

   i. The concentration of iron is higher in the brains of AD patients.

   ii. Copper is an important part of antioxidant enzymes, Cu/Zn SOD, and cytochrome-c oxidase and is found in lower concentrations in the brains of AD patients. However, copper ions are powerful promoters of peroxidation and have been linked to the exacerbation of oxidative damage in AD.\textsuperscript{228}

   iii. Zinc induces amyloid formation in humans and there is increasing evidence that high zinc concentrations can mediate neuronal death associated with other brain injuries.\textsuperscript{229}

   iv. Higher levels of aluminum have been measured in the cores of senile plaques in AD. Humans are constantly exposed to this metal through foods, beverages, medications, personal hygiene products, and cookware. It has been proposed that aluminum ions bind to membranes and cause a rearrangement of lipids that lead to propagation of lipid peroxidation. Although aluminum exists only in the trivalent form and is redox-inert, it is possible that it may contribute to lipid peroxidative damage in AD by escalating iron and copper induced free radical damage. An excellent review details the mechanisms that form the hypothesis that aluminum may exacerbate events associated with AD.\textsuperscript{230} Two of the pathological features of AD are neurofibrillary tangles and senile plaques. These plaques are described as localized areas of degenerating and swollen axons, neuritis, and glia surrounding a core of amyloid. These amyloid protein deposits in AD contain truncated products including $\beta$-amyloid. Several brain regions show massive neuronal loss in AD. Many \textit{in vitro} studies have confirmed a direct toxic effect of $\beta$ Amyloid on cultures of neurons. This $\beta$-amyloid toxicity is prevented by vitamin E and other antioxidants and mediated by hydrogen peroxide.\textsuperscript{231}
5.8 Role of Oxidation stress in depression

There is evidence for oxidative disturbances in major depression, as demonstrated by oxidative marker studies and those examining the antioxidant effect of antidepressants. XO catalyzes the oxidation of xanthine, a process that generates ROS, further SOD catalyses to superoxide and hydrogen peroxide, and RNS. XO-generated O & NS has been implicated in ischemia/reperfusion injury and multisystem organ failure. Where as higher concentration of SOD is found in the brain in depressed patients. There is evidence for increased antioxidant defences and increased ROS and RNS in patients with major depression. Throughout their lifespan depressed patients may be challenged with several depressive episodes, associated with (sub) chronic inflammatory responses and by inference with significantly increased ROS/RNS production. Increases in ROS/RNS and decreased antioxidant defences may cause oxidative and nitrosative modifications of cellular molecules, such as fatty acids, proteins and DNA. Consequently, O & NS may have detrimental effects on membrane fatty acids, the function and stability of proteins, and DNA damage and its repair mechanisms as well. If unchecked, this may result in apoptoisis, and in part explain the brain volumetric changes evident in depression. This paragraph aims to discuss findings on O & NS damage to different substrates.

SOD activities in depressed patients as compared to controls was found to less, although these differences did not reach significance. Selek reported lowered SOD activity in depressed patients. Herken also found lowered SOD levels in depressed patients. Sarandol, however, detected a significantly increased SOD activity in depression that was significantly correlated to the severity of illness. Andrazza et al found increased activity as well an increased SOD / GPX plus catalase ratio, in depressed patients. During the acute phase of illness, depressed patients had significantly higher activity levels of SOD1 as compared to healthy controls. During treatment with antidepressants the increased SOD1 levels normalized. Szuster-Ciesielska et al found significantly increased serum activity of SOD in depressed patients. Finally, in post-mortem brain tissue of patients with recurrent depressive disorder increased SOD activity has been
found in the prefrontal cortex. Increased SOD activity probably reflects an upregulated SOD system in defence against increased ROS and free radicals in depression. At first sight these results may seem contradictory. A factor that may explain the differences in outcome of these studies is stage or duration of illness. In bipolar disorder, there is evidence that there are stage related alterations in oxidative and inflammatory markers, which may be due to failure of compensatory mechanisms with the process of neuroprogression.

5.9 Antioxidant defenses

The antioxidant network is complex and includes both endogenous and diet derived molecules. Superoxide dismutase enzymes (SODs) remove $\text{O}_2^*$ by accelerating its conversion to $\text{H}_2\text{O}_2$ (eqn I). Human cells have a SOD enzyme containing manganese at its active site (MnSOD) in the mitochondria. A SOD with copper and zinc at the active site (CuZnSOD) is also present, but largely in the cytosol. Catalase enzymes convert $\text{H}_2\text{O}_2$ to water and $\text{O}_2$, but more important $\text{H}_2\text{O}_2$ removing enzymes in human cells are the glutathione peroxidases (GSHPX), one of the few classes of human enzymes that require selenium for their action. GSHPX enzymes remove $\text{H}_2\text{O}_2$ by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH (reduced nicotinamide adenine dinucleotide) as a source of reducing power. Thioredoxin-dependent systems are also essential to $\text{H}_2\text{O}_2$ removal and redox regulation within cells; thioredoxin reductase is a selenoprotein. Organisms are also careful to keep iron and copper safely protein bound whenever possible, so that the reaction shown in (eqn II) is prevented. In addition to enzymes, low-molecular-mass free radical scavengers exist. GSH can scavenge various reactive species (e.g. HOCl and ONOO) directly, as well as being a substrate for GSHPX enzymes. $\alpha$-Tocopherol (derived from the diet, as vitamin E) is the most important free radical scavenger within membranes. It can inhibit lipid peroxidation by scavenging peroxyl radical intermediates and so halting the chain reaction. Several other...
Antioxidants are present in the diet, including Ascorbate (vitamin C) and flavonoids.

Antioxidant defenses exist as a balanced coordinated system. Thus, although SOD is important to normal cell function, an excess of SOD in relation to activities of peroxide-metabolizing enzymes can be deleterious. This has been shown by transfecting cells with human cDNAs encoding SOD. The consequences of excess SOD may be relevant to the clinical condition known as Down syndrome, in which trisomy of chromosome 21 leads to elevated levels of CuZnSOD, the gene encoding which is located on this chromosome.

In the healthy human body, there is an approximate balance between production of reactive species and antioxidant defenses. Indeed, low levels of products of free radical attack on biomolecules are found even in healthy tissues. Antioxidant defenses do not completely prevent attack by reactive species, so that repair systems are also needed to minimize damage levels. Repair of oxidized DNA (e.g. removal of 8-hydroxyguanine residues) is especially important.

5.10 Antioxidants and their mechanism

Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms:

1. Scavenging species that initiate peroxidation
2. Chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides.
3. Quenching $\text{O}_2^*$ preventing formation of peroxides.
4. Breaking the autooxidative chain reaction, and/or
5. Reducing localized $\text{O}_2$ concentrations
Chain breaking antioxidants differ in their antioxidative effectiveness depending on their chemical characteristics and physical location within a food (proximity to membrane phospholipids, emulsion interfaces, or in the aqueous phase). The chemical potency of an antioxidant and solubility in oil influence its accessibility to peroxo radicals especially in membrane, micellar and emulsion systems, and the amphiphilic character required for effectiveness in these systems.\(^{246}\) Antioxidant effectiveness is related to activation energy, rate constants, oxidation–reduction potential, ease with which the antioxidant is lost or destroyed (volatility and heat susceptibility), and antioxidant solubility.\(^{245}\) In addition, inhibitor and chain propagation reactions are both exothermic. As the A:H and R:H bond dissociation energies increase, the activation increases and the antioxidant efficiency decreases. Conversely, as these bond energies decrease, the antioxidant efficiency increases. The most effective antioxidants are those that interrupt the free radical chain reaction. Usually containing aromatic or Phenolic rings, these antioxidants

**Figure 3.6 Mechanism reaction of free radicals and antioxidants**
donate H to the free radicals formed during oxidation becoming a radical themselves. These radical intermediates are stabilized by the resonance delocalization of the electron within the aromatic ring and formation of quinone structures. In addition, many of the phenolics lack positions suitable for molecular oxygen attack. Both synthetic antioxidants (BHA, BHT, and propyl gallate) and natural botanicals contain phenolics (flavonoids) function in this manner. Botanical extracts with antioxidant activity generally quench free radical oxygen with phenolic compounds as well. Because bivalent transition metal ions, Fe$^{2+}$ in particular, can catalyze oxidative processes, leading to formation of hydroxyl radicals, and can decompose hydroperoxides via Fenton reactions, chelating these metals can effectively reduce oxidation. Food materials containing significant amounts of these transition metals (red meat) can be particularly susceptible to metal-catalyzed reactions.

![Antioxidant groups and actions](image)

**Figure 3.7** Antioxidant groups and actions. SOD = Superoxide Dismutase, GPX = Glutathione Peroxidase, dotted lines = suppression.
5.11 Mechanism of Polyene antioxidants against oxidative stress

Highly reactive oxygen molecules are generated during normal cellular respiration and causes toxic injury. Oxygen in the presence of electrons can form the free radical superoxide (O$_2^-$). Superoxide can be rapidly converted by superoxide dismutase (SOD) to hydrogen peroxide which, in turn, can generate highly reactive hydroxyl radicals (OH•) when iron is present. Superoxide can also combine with nitric oxide to form hydroxyl and nitrogen dioxide radicals (NO$_2^-$) via peroxynitrite anion (OONO$^-$) and HOONO. Macromolecular damage will result if reactive oxygen molecules are not neutralized. Catalase and glutathione (GSH) remove ROS via enzymatic mechanisms that convert hydrogen peroxide to water and O$_2$. Polyene antioxidants have inherent property to prevent damage by scavenging ROS and lipid-peroxyl radicals by interacting them to form peroxide and hydroxyl charged moieties.

![Figure 3.8 Mechanism of polyene antioxidants as free radical scavengers](image)

Figure 3.8 Mechanism of polyene antioxidants as free radical scavengers
6. The Blood–Brain Barrier

The brain is sheltered from the changing metabolite concentrations in blood by a blood–brain barrier (BBB) that surrounds the central nervous system (CNS) including the spinal cord. The BBB is necessary to provide an optimal chemical environment for cerebral function. Several layers exist between blood and brain: capillary endothelial cells, a basement membrane consisting of type IV collagen; fibronectin and laminin that completely covers the capillaries; pericytes embedded in the basement membrane; and astrocyte processes that surround the basement membrane. Each of these layers could, potentially, restrict the movement of solutes. Endothelial cells were demonstrated to be the site of the BBB when it was observed that horseradish peroxidase could not pass the endothelial layer from either direction. Although Pappenheimer\textsuperscript{248} challenged this concept, arguing that astrocytes were a more likely site of the barrier, Crone\textsuperscript{249} definitely decided the issue by demonstrating that brain capillaries from amphibians, which have no surrounding layer of astrocytes, have high electrical impedance= 2,000 $\Omega \times \text{cm}^2$, which is indicative of a restriction to the movement of ions. The cerebral endothelium is now accepted as the site of the BBB in higher animals.

Cerebral capillary endothelial cells differ from other mammalian capillary endothelial cells; they have few cytoplasmic vesicles, more mitochondria, and a larger number of tight junctions between overlapping cells. The tight junctions inhibit paracellular movement, prevent membrane molecules from moving from one cell to another, and divide the membranes of the endothelial cells into two distinct sides, luminal (blood side) and abluminal (brain side). Different populations of both lipids and intrinsic proteins (e.g., transporters) exist on the luminal and abluminal sides. Therefore, hydrophilic nutrients must pass two sheaths of membrane, the combined characteristics of which determine which particles traverse the barrier and how quickly. Pappenheimer and Setchell\textsuperscript{248} recognized the implication of passing two membranes in series to gain entry to the CNS.
Passive diffusion of substances across the brain endothelial cells may occur and is dependent on lipophilicity and molecular weight. However, a large number of drugs that possess a favourable lipophilicity that normally should enable an easy transport across these cells are rapidly pumped back into the blood stream by extremely effective efflux pumps. These pump systems include multiple organic anion transporter (MOAT) and especially P-glycoprotein (Pgp) sometimes referred to as multi-drug resistance protein (mdr).

A number of attempts have been made to overcome the above barrier including osmotic opening of the tight junctions, use of prodrugs or carrier systems such as antibodies, liposomes, and nanoparticles. The opening of the tight junctions by osmotic pressure, however, is a very invasive procedure that also enables the entry of unwanted substances into the brain. Prodrugs take advantage of a higher lipophilicity enabling a better penetration and transport into and across the lipophilic endothelial barrier and/or of
a circumvention of the efflux-pump systems, but often the prodrug approach is not possible. The colloidal carriers, on the other hand, may take advantage of the biochemical transport systems that are also present in the BBB: The brain is dependent on the blood to deliver substrates as well as to remove metabolic waste. For this reason, carrier-mediated transport systems exist that enable the entry or the elimination of a variety of compounds including hydrophilic substances such as hexoses, amino acids, purine compounds, and mono-carbonic substances as well as lipoproteins including low density lipoprotein (LDL). Among these systems, for instance, the LDL-receptor and the transferrin transcytosis systems have been employed in the delivery of drugs by the above particulate colloidal drug delivery systems.

6.1 Mechanism of nanoparticle-mediated drug transport to the brain

A number of possibilities exist that could explain the mechanism of the delivery of the above mentioned substances across the BBB:

Mechanism. 1 An increased retention of the nanoparticles in the brain blood capillaries combined with an adsorption to the capillary walls. This could create a higher concentration gradient that would enhance the transport across the endothelial cell layer and as a result the delivery to the brain.

Mechanism. 2 A general surfactant effect characterized by a solubilization of the endothelial cell membrane lipids that would lead to membrane fluidization and enhanced drug permeability through the BBB.

Mechanism. 3 The nanoparticles could lead to an opening of the tight junctions between the endothelial cells. The drug could then permeate through the tight junctions in free form or together with the nanoparticles in bound form.

Mechanism. 4 The nanoparticles may be endocytosed by the endothelial cells followed by the release of the drugs within these cells and delivery to the brain.
Mechanism. 5  The nanoparticles with bound drugs could be transcytosed through the endothelial cell layer.

Mechanism. 6  The polysorbate 80 used as the coating agent could inhibit the efflux system, especially P-glycoprotein (Pgp).

All these mechanisms also could work in combinations. Among these mechanisms, mechanisms 1 and 2 are unlikely to contribute to the observed nanoparticle-mediated drug delivery to the brain to a major degree: loperamide, doxorubicin, and tubocurarine are known substrates for Pgp. It is very unlikely that the creation of a higher local concentration in the brain capillaries alone (mechanism 1) would be sufficient to overcome the action of the very effective efflux pumps located in the luminal membrane of the endothelial cells such as Pgp. A general surfactant effect (mechanism 2) can be ruled out by the experiments of Kreuter et al. in which the dalargin nanoparticles were overcoated with a number of different surfactants. Only overcoating with polysorbate 20, 40, 60, and 80 induced an antinociceptive effect, whereas the other surfactants, although being very effective solubilizing agents, were not able to transport dalargin into the brain in pharmacologically sufficient concentrations.

The opening of the tight junctions (mechanism 3) can be investigated by the measurement of the so-called inulin spaces by the Oldendorf method. A significant opening of the inulin spaces for instance by osmotic methods increases the space by factors of 10–20 (1000–2000%). The in vivo determination of the inulin spaces in defined brain regions of rats by Alyautdin et al. confirmed that the control inulin spaces obtained in that study were similar to the comparable vascular spaces obtained for the rat in other studies and species. In rats treated with polysorbate 80-coated nanoparticles the inulin spaces were increased by 10% after 10 min and 99% after 45 min. This increase would suggest that the coated nanoparticles were increasing the volume available to the intravascular inulin but were not significantly disrupting the blood-brain barrier. This increase could be due to a slight opening of the tight junctions, an upfolding of the cell membrane due to endocytotic events, or to an increase in fluid phase endocytosis of inulin associated with the internalisation of the nanoparticles. In this context it is interesting to note that the inulin spaces in rat brain structures are larger than
the plasma space determined in previous studies, suggesting that inulin finds a space in the brain greater than that available to larger tracers such as albumin and erythrocytes. In this study the fact that the inulin space also increased in non-blood-brain barrier structures such as the pituitary and the choroid plexus is a further indication that the increase in the spaces is the result of inulin entry into cells possibly by endocytosis. On the other hand, the rapid onset of the antinociceptive effects after injection of the polysorbate 80-coated nanoparticles was reported.

At present the most likely mechanism of nanoparticle-mediated transport of drugs to the brain is mechanism 4, endocytosis of the nanoparticles and release of the drug in these cells. Endocytosis of polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles with a variety of different labels by brain blood vessel endothelial cells has frequently been observed and in vitro been demonstrated by fluorescence and laser confocal microscopy. At an incubation temperature of 37°C only polysorbate overcoating led to a significant and rapid uptake, whereas without coating this uptake was minimal. The uptake of the polysorbate 80-coated nanoparticles was inhibited at 48°C or by a pretreatment with cytochalasin B, a potent phagocytic uptake inhibitor.

Adsorption of apolipoprotein E (apo E) on the surface of polysorbate 20, 40, 60 or 80-coated nanoparticles after incubation for 5 min in human citrate-stabilized plasma at 37°C. The particles were separated from the serum by centrifugation and the adsorbed plasma proteins desorbed and analyzed by 2-D PAGE (two dimensional polyacrylamide gel electrophoresis). Only after polysorbate 20, 40, 60, or 80 overcoating an apo E adsorption was detected whereas no apo E adsorption resulted after incubation of uncoated nanoparticles or overcoating with poloxamers 338, 407, Cremophor EL, or Cremophor RH40. Apo E does play an important role in the transport of LDL into the brain. Lipoproteins are of critical importance for the delivery of essential lipids to this organ. The presence of an LDL receptor in the BBB has been demonstrated and apo E and apo A-I containing particles have been detected in human cerebrospinal fluid. It is very possible that the polysorbate coated nanoparticles mimic LDL-particles after apo E adsorption following injection into the blood. In this way they could act as Trojan horses by interacting with the LDL receptor leading to their uptake by endocytic processes.
The role of polysorbates would be that of an anchor for apo E: Although preincubation of uncoated nanoparticles with apo E before the in vitro incubation in human plasma also led to a significant adsorptive apo E coating of the nanoparticles, without a polysorbate anchor this apo E layer was to a large degree displaced by other plasma components during plasma incubation. Accordingly, in vivo the antinociceptive effect of the dalargin nanoparticles was significantly lower if the nanoparticles were incubated in apo E alone without polysorbate prior to injection but it remained similar to coating with polysorbate 80 alone if the particles were coated with the polysorbate before the incubation with apo E.\textsuperscript{267, 268}

Interestingly and in contrast to what would be expected from these considerations, preincubation of the bovine brain endothelial cells used by Ramge with lipoprotein-deficient fetal calf serum increased the uptake in comparison to control cells with the polysorbate 80-coated nanoparticles.\textsuperscript{260} This poses the question, why was the uptake of the polysorbate 80-coated nanoparticles in vitro enhanced and not reduced after incubation of these cells with lipoprotein-deficient calf serum? Probably the cells were starved by this treatment leading to an up-regulation of the LDL-receptor. Residual lipoprotein could adsorb to the nanoparticles and possibly, as a result, the cells would take up these particles to a much higher degree.

Little is presently known about mechanism 5, transytosis of the nanoparticles. Of course, it is possible that after their endocytotic uptake the particles can be transcytosed through the brain blood vessel endothelial cells. In vitro transcytosis of LDL across the BBB was observed in the Cecchelli-Model by Dehouck et al.\textsuperscript{269} This process was totally blocked by the C7 monoclonal antibody that is known to interact with the LDL receptor. Furthermore, cholesterol depletion upregulated the expression of the LDL receptor in this model. Using the same in vitro model, transcytosis of dipalmitoyl phosphatidyl choline and cholesterol coated ionic crosslinked malto-dextrin nanoparticles of a size of about 60 nm was observed by Fenart et al.\textsuperscript{258} Hence, it is possible that the polysorbate-coated poly(butyl cyanoacrylate) nanoparticles also can be transcytosed.

The material used in most of the above experiments for overcoating, polysorbate 80, was shown to be able to inhibit Pgp.\textsuperscript{270} As mentioned above, this glycoprotein is present in the
brain endothelial cells and it is responsible for the multidrug resistance which represents a major obstacle to cancer chemotherapy. Hence, inhibition of this efflux pump located in the brain blood vessel endothelial cell also could be responsible for the nanoparticle-mediated transport of drugs to the brain. However, as shown in the in vivo experiments, polysorbate 80 added to the drug solutions in concentrations of 1% as used with the nanoparticles had no effect. On the other hand, the surfactant, of course, may be delivered to the brain endothelial cells more efficiently if it is adsorbed to the nanoparticles.

6.2 Lipoprotein Receptors

Apolipoprotein E (apoE) is a 34-kDa protein constituent of both very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL), which transports cholesterol and other lipids in the plasma and in the CNS. The lipoproteins complexes can be taken up in the brain through the recognition of apoE by specific receptors at the BBB, which include the low-density lipoprotein receptor (LDLR) and the LDLR-related protein (LRP). Taking into consideration that LRP has been reported to be highly expressed on endothelial brain microvessels, receptor-mediated transcytosis using NPs functionalized to bind this target has been exploited in several ways.

First, by exploiting the preferential absorption of ApoE on some types of NPs when in serum. This feature has been described for PBCA NPs coated with polysorbate 80, indeed displaying the ability to cross the BBB in vivo in animal models and for PEGylated PHDCA NPs, able to penetrate into brain endothelial cells in vitro. Taking advantage of such opportunity, the formation of a stable ApoE decoration of NPs surface by covalent binding has been utilized to functionalize albumin-NPs or liposomes that showed the capacity of enhancing BBB passage in vivo in rodent models.

It should be questioned whether other apolipoproteins may be employed for NPs functionalization to confer on them the ability to enhance brain drug delivery. In order to answer this question, in an investigation of Kreuter et al., PBCA NPs loaded with dalargin or loperamide were coated with different apo-lipoproteins, AII, B, CII, E, or J, coated or not with polysorbate 80, and then injected in mice and the drug effect on CNS...
was evaluated. The results showed that only NPs coated with apolipoprotein B or E were able to achieve an antinociceptive effect. This effect was significantly higher after polysorbate-precoating and apolipoprotein B or E-overcoating.\textsuperscript{277}

\textbf{Figure 3. 10 Mechanisms evidenced for the blood brain targeting}

The region of apoE that is critical for interaction with the LDL receptor resides between amino acid residues 140 and 160. Several studies with synthetic peptides have investigated the structural features of the LDLR-binding sequence of ApoE.\textsuperscript{278, 279} Laskowitz et al.\textsuperscript{272} derived an apoE-mimetic peptide from amino acids 133–149, named COG133, which retains its biological activity in vitro and in vivo. This capacity is displayed also by a fragment of 26 amino acids from ApoE4.\textsuperscript{280}

It has also been reported that the tandem dimer (141–155), is recognized by the LDLR, in contrast to the monomeric peptide (141–155).\textsuperscript{281} Also a shorter sequence of this peptide, the tandem dimer (141–150), retains this ability.\textsuperscript{282} Probably as a consequence of these observations, sterically stabilized liposomes were functionalized only with ApoE tandem
dimer peptide (141–150)$_2$, but not with the monomer, and were shown to be efficiently taken up by rat brain capillary endothelial cells.$^{283}$ Interestingly, in spite of the theoretical premises based on receptor-mediated endocytosis, liposomes tagged with (141–150)$_2$ were nonselectively internalized into cultured BBB cells, and clathrin- or caveolin-dependent endocytosis was not demonstrable.$^{284}$

However, in a successive investigation the ApoE-derived peptide monomer 141–150 was utilized by Re et al.$^{285}$ in comparison with the dimer, for the functionalization of liposomes entrapping a radioactive derivative of curcumin. The investigation showed a higher uptake of the drug by human endothelial cells and an enhancement of permeability of the drug across an in vitro model of the BBB made with the same cells, when using monomer-functionalized liposomes.

![Figure 3. Mechanism of drug delivery across the blood brain barrier by polysorbate coated Nanoparticles](image-url)
7. Nanoparticles as Drug Delivery System

Nanoparticles are solid submicron colloidal polymeric carrier systems ranging in size from 1 to 1000 nm that are utilized as drug delivery agents. Drugs in nanoparticles may be present in the form of a solid solution or dispersion or be adsorbed to their surface or may be chemically attached. Depending upon the method of preparation, either nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymers such as poly (ethylene glycol) (PEG) known as long circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period, to target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes.\textsuperscript{286, 287} The advantages of using nanoparticles as a drug delivery system include:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.

2. They control and sustain the release of drugs during their transportation and at the site of localization, altering organ distribution and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and to reduce its side effects.

3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction. For preserving the drug activity, this is an important factor.

4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or by the use of magnetic guidance.

5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The factors that govern the selection of materials for the preparation of nanoparticles include the size of
nanoparticles required, inherent properties of the drug e.g., aqueous solubility and stability; surface characteristics such as charge and permeability, degree of biodegradability, biocompatibility and toxicity, desired drug release profile and antigenicity of the final product.\textsuperscript{288}

Nanoparticles have been prepared generally by various methods like dispersion of preformed polymers, polymerization of monomers and by ionic gelation or coacervation of hydrophilic polymers. However, other methods such as supercritical fluid technology\textsuperscript{289} and particle replication in non-wetting templates\textsuperscript{290} have also been described in the literature for production of nanoparticles.

7.1 Limitations of CNS Drug targeting by nanoparticles

Initially, the intravenous administration of nanoparticles did not prove to be successful in targeting the drugs to the brain. Failure of nanoparticles to reach the CNS in appreciable quantity was attributed to their uptake by the reticulo-endothelial system (RES). The RES significantly removes a large portion (up to 80-85%) of nanoparticles from the vascular space, thus limiting their exposure to the cerebrovasculature and resulting in decreased drug concentration in the brain.\textsuperscript{291} The problem of rapid uptake of nanoparticles by the RES was partially solved by coating them with surfactants.\textsuperscript{292} Primary surfactants used include poloxamine 908 and polysorbate-80. Reduction in the uptake of nanoparticles by the RES results in an increased residence time of the drug in circulation and an increased uptake in non-RES organs. Poloxamine 908 used as a surfactant on hydrophobic nanoparticles was shown to reduce RES uptake in the liver when compared with uncoated drug (72% vs. 19%). A significant reduction of nanoparticle uptake by the spleen, lungs and bone marrow has also been reported.\textsuperscript{293} Similarly, Polysorbate-80 has been shown to be effective in minimizing uptake by the RES.\textsuperscript{294}

7.2 Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are aqueous colloid-al dispersions, the matrix of which comprises of solid biodegradable lipids.\textsuperscript{295} SLNs combine the advantages and avoid the drawbacks of several colloidal carriers of its class such as physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability.\textsuperscript{296}
SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonary, rectal) have been developed and thoroughly characterized in vitro and in vivo.\textsuperscript{297}

### 7.3 Advantages of SLN

i. Use of biodegradable physiological lipids which decreases the danger of acute and chronic toxicity and avoidance of organic solvents in production methods.\textsuperscript{298}

ii. Improved bioavailability of poorly water soluble molecules.\textsuperscript{299} Site specific delivery of drugs, enhanced drug penetration into the skin via dermal application. Possibility of scaling up.

iii. Protection of chemically labile agents from degradation in the gut and sensitive molecules from outer environment, SLNs have better stability compared to liposome

iv. It enhance the bioavailability of entrapped bioactive and chemical production of labile incorporated compound.

### 7.4 Disadvantages of SLN

i. Poor drug loading capacity,

ii. Drug expulsion after polymeric transition during storage

iii. Relatively high water content of the dispersions (70-99.9%).\textsuperscript{300}

### 7.5 Preparation techniques of SLN

**High shear homogenization (HSH)**

Initially used for the production of solid lipid nanoemulsions, this method is reliable. It involves high pressure homogenization which pushes the liquid with high pressure (100-2000 bar) through a narrow gap ranging a few microns. The fluid accelerates to a very short distance at very high viscosity of over 1000 km/h. Very high shear stress and cavitation forces disrupt the particles down to submicron range. As low as 5% to as high
as of 40% lipid content has been investigated. Two general approaches to achieve HSH are hot homogenization and cold homogenization.

Hot homogenization is generally carried out at temperatures above the melting point of the lipid. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high shear mixing device. The resultant product is hot o/w emulsion and the cooling of this emulsion leads to crystallization of the lipid and the formation of SLNs. Smaller particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase. However, high temperature leads to the degradation rate of the drug and the carrier. Increasing the homogenization temperature or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles. Generally, 3-5 homogenization cycles at a pressure of 500-1500 bar are used.

Cold homogenization has been developed to overcome the temperature related degradation problems, loss of drug into the aqueous phase and partitioning associated with hot homogenization method. Unpredictable polymeric transitions of the lipid due to complexity of the crystallization step of the nanoemulsion resulting in several modifications and/or super cooled melts. Here, drug is incorporated into melted lipid and the lipid melt is cooled rapidly using dry ice or liquid nitrogen. The solid material is ground by a mortar mill. The prepared lipid microparticles are then dispersed in a cold emulsifier solution at or below room temperature. The temperature should be regulated effectively to ensure the solid state of the lipid during homogenization. However, compared to hot homogenization, larger particle sizes and a broader size distribution are typical of cold homogenization samples.

**Ultrasonication**

Ultrasonication or high speed homogenization is another method for the production of SLNs. The advantage of this method is that the equipment used is commonly available at lab scale. However, this method suffers from problems such as broader size distribution ranging into micrometer range. Potential metal contaminations, physical instability like particle growth upon storage are other drawbacks associated with this technique.
Microemulsion based SLN preparation

Gasco and coworkers\textsuperscript{303} developed SLNs based on the dilution of microemulsions. These are made stirring an optically transparent mixture at 65-70°C which is typically composed of a low melting fatty acid like stearic acid, an emulsifier (e.g. polysorbate 20, polysorbate 60, soyaphosphatydylcholine and taurodeoxycholic acid sodium salt), co-emulsifiers (e.g. butanol, sodium monooctylphosphate) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring.\textsuperscript{304} Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion. The SLN dispersion can be used as granulation fluid for transferring in to solid product like tablets and pellets by granulation process, but in case of low particle content too much of water need to be removed. The nanoparticles were produced only with solvents which distribute very rapidly into the aqueous phase (acetone), while larger particle sizes were obtained with more lipophilic solvents.\textsuperscript{305}

Supercritical Fluid technology

This is a novel technique recently applied for the production of SLNs.\textsuperscript{306} A fluid is termed supercritical when its pressure and temperature exceed their respective critical value. The ability of the fluid to dissolve compounds increases. This technology comprises of several processes for nanoparticle production such as rapid expansion of supercritical solution (RESS), particles from gas saturated solution (PGSS), aerosol solvent extraction solvent (ASES), supercritical fluid extraction of emulsions (SFEE). The advantages of this technique includes avoidance of the use of solvents, particles obtained as a dry powder, instead of suspensions, requires mild pressure and temperature conditions. Carbon dioxide solution is the good choice as a solvent for this method.\textsuperscript{307}

Solvent emulsification/evaporation

For the production of nanoparticle dispersions by precipitation in o/w emulsions, the lipophilic material is dissolved in water-immiscible organic solvent (cyclohexane) that is emulsified in an aqueous phase.\textsuperscript{308} Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean
diameter of the obtained particles was 25 nm with cholesterol acetate as model drug and leci-thin/sodium glycocholate blend as emulsifier. The reproducibility of the result was confirmed by Siekmann and Westesen\textsuperscript{309} who produced the cholesterol acetate nanoparticles of mean size 29 nm.

**Solvent emulsification-diffusion**

SLNs can also be produced by solvent emulsification-diffusion technique. The mean particle size depends upon lipid concentration in the organic phase and the emulsifier used. Particles with average diameters of 30-100 nm can be obtained by this technique. Avoidance of heat during the preparation is the most important advantage of this technique. Here, the lipid matrix is dissolved in water-immiscible organic solvent followed by emulsification in an aqueous phase. The solvent is evaporated under reduced pressure resulting in nanoparticles dispersion formed by precipitation of the lipid in aqueous medium.\textsuperscript{310, 311}

**Double Emulsion**

In this method, the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion. Li \textit{et al.} prepared solid lipid nanoparticles loaded with bovine serum albumin (BSA) using double emulsion method.\textsuperscript{312}

**Spray Drying**

It is an alternative technique to lyophilization in order to transform an aqueous SLN dispersion into a drug product. This is a cost-effective method than lyophilization and recommends the use of lipid with melting point \(>70^\circ\text{C}\). This method causes particle aggregation due to high temperature shear forces and partial melting of the particle. According to Freitas and Muller\textsuperscript{313} best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixtures (10/90 v/v).
**Solvent injection technique**

Here, the solid lipid is dissolved in water miscible solvent. The lipid solvent mixture is injected into stirred aqueous phase with or without surfactant. Finally, the dispersion filtered to remove excess lipid. Emulsion within the aqueous phase helps to produce lipid droplets at the site of injection and stabilize SLNs until solvent diffusion gets completed. Mishra\textsuperscript{314} prepared and evaluated SLNs using Solvent injection method for delivery of Hepatitis B surface antigen for vaccination using subcutaneous route.

### 7.6 Polymeric Nanoparticle

The polymeric nanoparticles (PNPs) are prepared from biocompatible and biodegradable polymers in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.\textsuperscript{315} The field of polymer nanoparticles (PNPs) is quickly expanding and playing an important role in a wide spectrum of areas ranging from electronics, photonics, conducting materials, sensors, medicine, biotechnology, pollution control and environmental technology. PNPs are promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to specific target, such an advantage improves the drug safety.\textsuperscript{316} Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometer size promotes effective permeation through cell membranes and stability in the bloodstream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into unique nanoparticle constructs with many potential medical applications.\textsuperscript{317} Several methods have been developed during the last two decades for preparation of PNPs, these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer or ionic gelation method.
7.7 Advantages of polymeric nanoparticles\textsuperscript{318, 319}

i. Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.

ii. They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.

iii. Delivers a higher concentration of pharmaceutical agent to a desired location.

iv. The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives, and delivery of targeted antibiotics.

v. Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

7.8 Polymers used in preparation of polymeric nanoparticles

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible.

Table 3.6 Polymers use for preparation of polymeric nanoparticles

<table>
<thead>
<tr>
<th>Material</th>
<th>Full name</th>
<th>Abbreviation or Commercial name*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic homopolymers</td>
<td>Polylactide</td>
<td>PLA</td>
</tr>
<tr>
<td></td>
<td>Poly(lactide-co-glycolide)</td>
<td>PLGA</td>
</tr>
<tr>
<td></td>
<td>Poly(ε-caprolactone)</td>
<td>PCL</td>
</tr>
<tr>
<td></td>
<td>Poly(isobutyrylcyanoacrylate)</td>
<td>PICBA</td>
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<td></td>
<td>Poly(isohexylcyanoacrylate)</td>
<td>PIHCA</td>
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<tr>
<td></td>
<td>Poly(n-butyrylcyanoacrylate)</td>
<td>PBCA</td>
</tr>
<tr>
<td></td>
<td>Polyacrylates and polymethacrylates</td>
<td>Endragit*</td>
</tr>
<tr>
<td>Natural polymers</td>
<td>Chitosan</td>
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<td></td>
<td>Alginate</td>
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<td>Gelatin</td>
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<td></td>
<td>Albumin</td>
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<tr>
<td>Copolymers</td>
<td>Polylactide-poly(ethylene glycol)</td>
<td>PLA-PEG</td>
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<tr>
<td></td>
<td>Poly(lactide-co-glycolide)-poly(ethylene glycol)</td>
<td>PLGA-PEG</td>
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<tr>
<td></td>
<td>Poly(ε-caprolactone)-poly(ethylene glycol)</td>
<td>PCL-PEG</td>
</tr>
<tr>
<td></td>
<td>Poly(hexadecylcyanoacrylate-co-poly(ethylene glycol) cyanoacrylate)</td>
<td>Poly(HDCA-PEGCA)</td>
</tr>
<tr>
<td>Colloid stabilizers</td>
<td>Dextran</td>
<td></td>
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<tr>
<td></td>
<td>Pluronic F68</td>
<td>F68</td>
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<tr>
<td></td>
<td>Poly(vinyl alcohol)</td>
<td>PVA</td>
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<tr>
<td></td>
<td>Copolymers (see above)</td>
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<tr>
<td></td>
<td>Tween\textsuperscript{©} 20 or Tween\textsuperscript{©} 80</td>
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</table>
7.9 **Mechanisms of drug release**

i. The polymeric drug carriers deliver the drug at the tissue site by any one of the three general physico-chemical mechanisms.

ii. By the swelling of the polymer nanoparticles by hydration followed by release through diffusion.

iii. By an enzymatic reaction resulting in rupture or cleavage or degradation of the polymer at site of delivery, thereby releasing the drug from the entrapped inner core.

iv. Dissociation of the drug from the polymer and its de-adsorption/release from the swelled nanoparticles.

7.10 **Preparation techniques of Polymeric nanoparticles**

The properties of PNPs have to be optimized depending on the particular application. In order to achieve the properties of interest, the mode of preparation plays a vital role. Thus, it is highly advantageous to have preparation techniques at hand to obtain PNPs with the desired properties for a particular application. Different techniques like polymerization, preformed polymers, or ionic gelation etc are used.

Dispersion of drug in preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA), poly (D, L-glycolide) (PLG), poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA). These can be accomplished by different methods described below.

1. Solvent evaporation
2. Nanoprecipitation
3. Emulsification/solvent diffusion
4. Salting out
5. Dialysis
6. Supercritical fluid technology (SCF)

**Solvent evaporation**

Solvent evaporation was the first method developed to prepare PNPs from a. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, dichloromethane and chloroform preformed polymer were widely used, but are now replaced with ethyl acetate which has a better toxicological profile. The
emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. In the conventional methods, two main strategies are being used for the formation of emulsions, the preparation of single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods utilize high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized. Lemoine et al. prepared PLGA nanoparticles of about 200nm by utilizing dichloromethane 1.0% (w/v) as the solvent and PVA or Span 40 as the stabilizing agent. Song et al. prepared nanoparticles of PLGA with a typical particle size of 60–200nm by employing dichloromethane and acetone (8:2, v/v) as the solvent system and PVA as the stabilizing agent. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

**Nanoprecipitation**

Nanoprecipitation is also called solvent displacement method. It involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant. The polymer generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension. To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent that is also a non solvent of the polymer. The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for
lipophilic drugs when nanocapsules are prepared. The usefulness of this simple technique is limited to water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. Then, even though some water-miscible solvents produce certain instability when mixed in water, spontaneous emulsification is not observed if the coalescence rate of the formed droplets is sufficiently high. Although, acetone/dichloromethane (ICH, class 2) are used to dissolve and increase the entrapment of drugs, the dichloromethane increases the mean particle size and is considered toxic. This method is basically applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase, and it is not an efficient means to encapsulate water-soluble drugs. This method has been applied to various polymeric materials such as PLGA, PLA, PCL, and poly (methyl vinyl ether-comaleic anhydride) (PVM/MA). This technique was well adapted for the incorporation of cyclosporin A, because entrapment efficiencies as high as 98% were obtained. Highly loaded nanoparticulate systems based on amphiphilic h-cyclodextrins to facilitate the parenteral administration of the poorly soluble antifungal drugs Bifonazole and Clotrimazole were prepared according to the solvent displacement method.\footnote{328}

**Emulsification/solvent diffusion (ESD)**

This is a modified version of solvent evaporation method. The encapsulating polymer is dissolved in a partially water soluble solvent such as propylene carbonate and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. In fact, to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partly miscible with water or with another organic solvent in the opposite case. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. This technique presents several advantages, such
as high encapsulation efficiencies (generally >70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs. Several drug-loaded nanoparticles were produced by the ESD technique, including mesotetra(hydroxyphenyl)-porphyrin-loaded PLGA (p-THPP) nanoparticles, doxorubicin-loaded PLGA nanoparticles, plasmid DNA-loaded PLA nanoparticles, coumarin-loaded PLA nanoparticles, indocyanine, cyclosporine (Cy-A)-loaded gelatin and cyclosporin (Cy-A)-loaded sodium glycolate nanoparticles.

**Salting out**

Salting out is based on the separation of a water miscible solvent from aqueous solution via a salting out effect. The salting out procedure can be considered as a modification of the emulsification/solvent diffusion. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres.

**Dialysis**

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed PN. Polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cut off. Dialysis is performed against a non-solvent miscible with the former miscible. The displacement of the solvent inside the membrane
is followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles.

**Supercritical fluid technology**

The need to develop environmentally safer methods for the production of PNP has motivated research on the utility of supercritical fluids as more environmental friendly solvents, with the potential to produce PNPs with high purity and without any trace of organic solvent. Supercritical fluid and dense gas technology are expected to offer an interesting and effective technique of particle production, avoiding most of the drawbacks of the traditional methods.

**Table 3.7 Preparation techniques of polymeric nanoparticles**

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>By using a colloidal mill</td>
<td>Production of well characterized emulsions, uniform size. Easy to scale-up</td>
<td>High energy for the emulsification process</td>
</tr>
<tr>
<td>Emulsification–solvent evaporation</td>
<td>Possibility to encapsulate both hydrophilic and lipophilic drugs</td>
<td>Possible coalescence of the nanodroplets during the evaporation process</td>
</tr>
<tr>
<td>Emulsification–solvent diffusion</td>
<td>Control of nanoparticle size. Easy to scale-up</td>
<td>High volumes of water to be eliminated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leakage of water-soluble drug into the saturated-aqueous external phase</td>
</tr>
<tr>
<td>Emulsification–reverse salting-out</td>
<td>Minimal stress to fragile drugs. High loading efficiency. Easy to scale-up</td>
<td>Possible incompatibility between the salts and drugs. Purification needed to remove electrolytes</td>
</tr>
<tr>
<td>By gelation of emulsion droplets</td>
<td>Possibility to use natural macromolecules. hydrophilic and biocompatible</td>
<td>Limited to the encapsulation of hydrophilic drugs</td>
</tr>
<tr>
<td>Polymerization of alkyl cyanoacrylates</td>
<td>Easy method to obtain core-shell tuned nanoparticles. Control of nanoparticle size</td>
<td>Possible reaction between the drug and CeVI in the case of radical emulsion polymerization. Purification needed</td>
</tr>
<tr>
<td>Interfacial polycondensation</td>
<td>Low concentrations of surfactants. Modulation of the nanocapsule thickness by varying the monomer concentration</td>
<td>Limited to the encapsulation of lipophilic drugs. Purification needed</td>
</tr>
</tbody>
</table>
8. Nanoparticles of natural phytoconstituents

Nanoparticles are efficient delivery systems for the delivery of both hydrophilic and hydrophobic drugs. Controlled and sustained delivery of the paclitaxel nanoparticle is observed with reduction in toxic effects. Curcumin obtained from the rhizomes of turmeric (Curcuma longa) has the anticancer activity but it is limited due to its poor aqueous solubility which led to the poor bioavailability. Nanoparticles containing Curcumin was prepared by using the mixture of crosslinked and random copolymers of Nisopropylacrylamide(NIPAAM) with N vinyl-2-pyrrolidone (VP) and polyethylene glycol monoacrylate (PEG-A). In vitro release studies revealed 40% curcumin release in 24 hours in phosphate buffer at physiological pH and no tissue related toxicity was observed even at 20-fold void range of nanoparticles. The dispersion of nanocurcumin in aqueous media proved them as potential carrier for the treatment of the cancer as compared to free drug. Cuscuta chinensis is a Chinese drug containing flavonoids and lignins as active ingredients, used for nourishment of liver and kidney. Poor aqueous solubility of active ingredients led to the poor absorption upon oral administration. Cuscuta chinensis nanoparticles were prepared by using nanosuspension method and compared with Cuscuta chinensis ethanolic extract for hepatoprotective and antioxidant effects upon oral administration. An oral dose of Cuscuta chinensis nanoparticles at 25 and 50 mg/kg showed almost similar hepatoprotective activity as that of ethanolic extract of Cuscuta chinensis at 125 and 250 mg/kg, fivefold reduction in dose was observed with Cuscuta chinensis nanoparticles. Triptolide is known for the antiinflammatory, immunosuppressive, anti-
fertility and antineoplastic activities. The aqueous solubility of the drug is very poor and it shows some of the undesirable toxic effects. Nanoparticles and microemulsions containing triptolide were prepared and evaluated for anti inflammatory activity. The solid lipid nanoparticle formulation showed more anti-inflammatory activity than the microemulsion when evaluated in the rat paw oedema model.\textsuperscript{340} Nanocapsules of \textit{Zedoary} turmeric oil was found to produce increased hepatoprotective and anticancer effect as they showed improved stability and increased drug loading.\textsuperscript{341} Quercetin nanoparticles were prepared by precipitation technique. Increased antioxidant activity was achieved via improved released characteristics of the drug (74 times higher drug release).\textsuperscript{342} Taxol loaded nanoparticles were produced by emulsion solvent evaporation method and enhanced bioavailability and sustained release of drug was observed with nanoparticles.\textsuperscript{343} Solid lipid nanoparticles of silibinin were prepared by using hand shaking method. The solid lipid nanoparticles show the hepatoprotective effects as well as increase in the bioavailability due to increase in the circulation time and solubility of the silybinin.\textsuperscript{344} Inclusion of paclitaxel and doxorubicin to nanoparticles by using brij 78 surfactant led to the inhibition of P-gp mediated drug resistance and hence increase in the anticancer activity of the drugs.\textsuperscript{345}

\textbf{Table 3.8 Prepared nanoparticles of some bioactive phytoconstituents.}

<table>
<thead>
<tr>
<th>Name of Bioactive component/ Plant</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>Reduction in side effects</td>
<td>337</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Increase in solubility</td>
<td>338</td>
</tr>
<tr>
<td>\textit{Cuscuta chinensis}</td>
<td>Increase in solubility</td>
<td>339</td>
</tr>
<tr>
<td>Triptolide</td>
<td>Increase in solubility</td>
<td>340</td>
</tr>
<tr>
<td>Zedoary turmeric oil</td>
<td>Increased stability and drug loading</td>
<td>341</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Increased drug release and antioxidant effect</td>
<td>342</td>
</tr>
<tr>
<td>Taxol</td>
<td>Sustained release</td>
<td>343</td>
</tr>
<tr>
<td>Silybinin</td>
<td>Increase in circulation time</td>
<td>344</td>
</tr>
<tr>
<td>Paclitaxel and Doxorubicin</td>
<td>Inhibition of resistance</td>
<td>345</td>
</tr>
</tbody>
</table>
9. Role of Differential scanning calorimetry and X-ray diffractography in characterization of nanoparticles

Thermal analysis is used to analyze the incorporation of drugs into nanoparticles via examining enthalpy change.\textsuperscript{346} Liposomes have been used to penetrate skin for drug delivery and localized drug delivery.\textsuperscript{347} DSC is one of the primary tools used for the characterization of the matrix state, with polymorphism and drug incorporation in lipid dispersions.\textsuperscript{348} Nanoparticles tend to have a decreased melting temperature compared to bulk material that is not in the nanometer size.\textsuperscript{348} Lipid polymorphism is commonly found in lipid nanoparticle dispersions with various components affecting molecular packing, which is reflected in the different melting points and enthalpies.\textsuperscript{348} Furthermore, the smaller radius prevents optimal lipid packing of the lipid acyl chains, thus lowering the energy required for the phase transitions. Broadened profiles are usually attributed to the addition of multiple different lipid components, as well as size differences.\textsuperscript{348, 349}

Analysis of drug loading efficiencies is quite complicated, as the drug typically interacts with the lipids inducing a shift in the phase transition temperature.\textsuperscript{348} Moreover, the enthalpy of the transition may also be reduced as a population of lipids is interacting with the drug solubilized in the matrix.\textsuperscript{348} This can easily be used to identify if the drug is miscible in the melted state of the liposome. Most studies presume changes to the lipid thermogram and a negative shift of the matrix lipid $T_m$ to be a sign of drug incorporation. However, in some cases it has been reported that decreases in enthalpy can be attributed to lipid dissolution or aggregation of drug molecules within the nanoparticles.\textsuperscript{348}

Improved efficacy of different drugs has been studied using nanoparticle delivery systems.\textsuperscript{350} A potent cancer fighting drug, Paclitaxel, has difficulties in administration, due to poor solubility in water and with excipients. Nanoparticles composed of biodegradable polymers with poly(lactic-co-glycolic acid) have been used to encapsulate the drug within the nanoparticles, using emulsifiers such as cholesterol and phospholipids.\textsuperscript{350} DSC allowed for comparison of the thermodynamic properties, as the
Tm of Paclitaxel and the nanoparticle carriers were analyzed, to screen for undesirable changes to the drug. DSC was also used to record the transition of non-steroid anti-inflammatory drugs (NSAIDs) from a crystalline to an amorphous state upon encapsulation in polyethylene glycol (PEG), a solid drug carrier, accompanied by a decrease in endothermic transition over time and progressive scanning. Similar studies were performed with solid lipid nanoparticles (SLN) prepared from oil-water microemulsions to encapsulate the drug diazepam. Solid lipid nanoparticles are emerging as a potential application in drug delivery, due to their low toxicity and their ability to maximize drug incorporation for secondary and tertiary drug targeting. Thermograms of crystalline diazepam and the drug loaded SLN particle, showed that the melting peak for the drug was not observed in the loaded nanoparticles, indicating an amorphous solid in the SLN. Moreover, the solid state particles can exist in polymorphs, pseudopolymorphs, and even amorphous solids. DSC melting profiles are essential for identifying the state of the drug, which can significantly influence bioavailability, stability, and water content. This is essential for determining the proper state, for the active pharmaceutical ingredient.

Relating to drug stability, the lipids in SLNs are considered excipients, with different lipids and surfactants studied and presented in a recent review. Thermotropic analysis of the SLN particles indicated that the chemical stability of the lipid is not affected during formation with a low level of degradation (2-5%) for the majority of lipids and a maximum of 10% reached after 24 months. However, other lipid excipients such as lecithin have shown strong decomposition, minimizing its potential in SLN. Structural properties and thermodynamic characteristics of nanocrystallization have been studied in detail. In addition, DSC was used to analyze stability and drug dissolution from nanostructure lipid carriers (NLC) composed of a solid lipid matrix with a liquid lipid nanocompartment core. Drug release from three-dimensional polymer hydrogel systems is a growing field in biomedical drug delivery. Site-specific targeting and release increases the bioavailability. Thus, it is important for pharmaceutical testing, to
understand the interaction between nanoparticle carriers and drugs as well as nanoparticles and biological membranes. Different nanoparticle polymers and various cross-linkers have been used to modulate temperature-dependent drug release in vitro and some have been shown to obstruct drug diffusion and incorporation.358

X-ray diffraction (XRD) method for characterizing the crystal structures. Evaluation of crystal structure is generally important even for the nanoscale materials, the properties of which might be affected by the structures of several to several hundred nanometer scale, while the crystal structures are specified by the arrangement of atoms separated by about 0.1 nm. The XRD is based on the measurements of X-ray intensities scattered by the statistically distributed electrons belonging to the atoms in the material. The arrangement of atoms or the population of electrons is determined by analysis of angular dependence of scattered X-ray.359 The XRD method is suitable to determine the crystal structures by analyzing the positions and intensities of diffraction peaks typically observed for the well-crystallized material in the range of diffraction angle from 10 to 150°. The method is also used for evaluating the microstructures (crystallite size and microstrain) by analyzing the width and the shape of the peak profiles. The XRD method is sometimes called as wide angle X-ray diffraction (WAXD) method. The diffraction angle $2\theta$ the interplanar distance $d$ and X-ray wavelength are connected with each other by the Bragg's law.359

$$n \lambda = 2d \sin \theta$$

Figure 3.12 Diagrammatic representation of Braggs equation concept
10. Role of zeta potential and polydispersity in nanoparticle stability

Zeta Potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticle surface. This double layer of ions travels with the nanoparticle as it diffuses throughout the solution. The electric potential at the boundary of the double layer is known as the Zeta potential of the particles and has values that typically range from +100 mV to -100 mV. The magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticles with Zeta Potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions. Zeta Potential is an important tool for understanding the state of the nanoparticle surface and predicting the long term stability of the nanoparticle.

![Diagram of Zeta Potential](image)

**Figure 3.13 Development of Zeta potential in nanoparticles**

The particle motion due to the applied electric field is measured by light scattering. The particles are illuminated with laser light and therefore the particles scatter light. The
frequency of the scattered light is a function of particle velocity due to the Doppler shift. This explains another name for this technique: laser Doppler electrophoresis. A second beam of light (the reference beam) is mixed with the scattered beam in order to sensitively extract the frequency shift in the scattered light.363

From the known applied electric field and measured particle velocity, the particle mobility is readily determined. Zeta potential is then calculated from mobility by using a model, the most common of which is the Smoluchowski model. The only parameters required for determining zeta potential are liquid dielectric constant, refractive index, and viscosity. This makes the technique rapid and reliable.364

The Henry equation365 is then used to calculate the zeta potential, \( z \):

\[
U_E = \frac{2\varepsilon z f(ka)}{3\eta}
\]

- \( z \) Zeta potential.
- \( U_E \) Electrophoresis mobility.
- \( \varepsilon \) Dielectric constant.
- \( \eta \) Viscosity.
- \( f(ka) \) Henry’s function.
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