Nutrition 65 (2019) 138–157

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Review article

Fatty acids as essential adjuvants to treat various ailments and their role in drug delivery: A review

Aakash Katdare B. Pharm, MS. Pharm, Shreya Thakkar B. Pharm, M. Pharm, Shivshankar Dhepale B. Pharm, MS. Pharm, Dignesh Khunt B. Pharm, M. Pharm, Manju Misra B. Pharm, M. Pharm, Ph.D. *

Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Ahmedabad, India

ARTICLE INFO

Article History:
Received 23 May 2018
Received in revised form 1 February 2019
Accepted 20 March 2019

Keywords:
Polyunsaturated FA
FA
Lipids
Oils
Penetration enhancers
Blood–brain barrier

ABSTRACT

Since the discovery of fatty acids, they have carved their own niche as vital adjuvants in drug delivery and as treatment for various diseases. The literature has repeatedly described the essential role of various fatty acids in treating a wide range of diseases and disorders, from central nervous system diseases to wound healing. The use of fatty acids has expanded to many horizons and in recent decades they have gained importance as drug delivery adjuvants in addition to their auxiliary benefits in treating various diseases. Although fatty acids aid in solving both formulation-based and therapeutic challenges, they have never been viewed as dual agents by modern scientific literature. The aim of this review was to provide this perspective and combine the very discreet literature about fatty acids, which includes their role as therapeutic adjuvants and drug delivery agents. It gives insights on the use of fatty acids in treating the diseases of the eye, skin, central nervous system, viral diseases, and so on. The review further discusses how the structure of fatty acids plays an important role in therapeutic activity and affects formulation stability.

Introduction

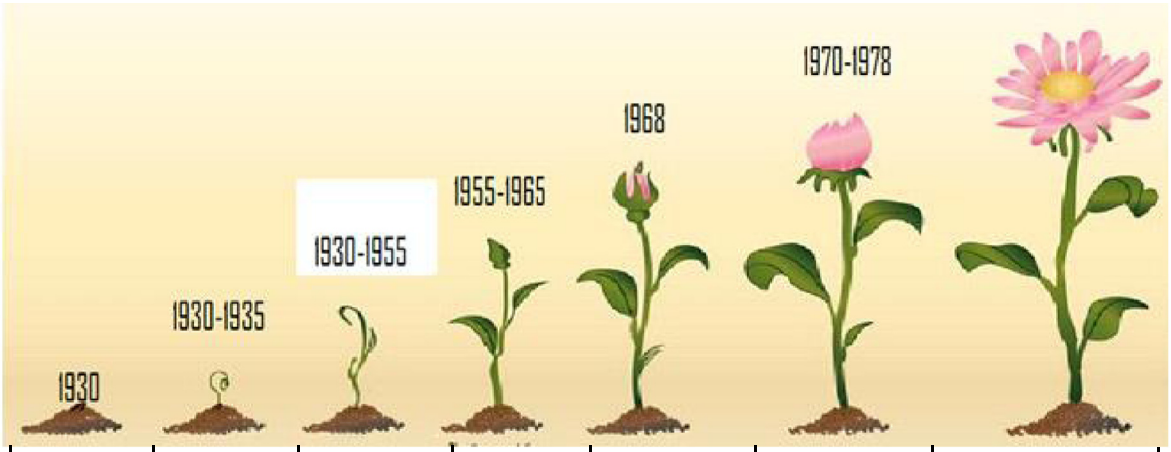
The value of lipids and dietary fats has been undervalued since the beginning of the 19th century, when they were considered only as an energy source rather than as an essential nutrient whose deficiency may cause any kind of disease [1]. The path of progress in our understanding of lipids and dietary fats, from thier discovery to their usage as essential adjuvants, has been a bumpy one. The earlier view about fatty acids (FAs) had not transcended their role as a mere energy source until a new paradigm of fat-free experimental methodology was discovered, which eventually led to identification of FAs as essential for the proper functioning of the human body. Subsequently, a study conducted on rats observed that the rats had suffered from a deficiency that led to scaly skin and other problems that were not resolved despite vitamin supplementation. This eventually led to discovery of the important role that linoleic acid played in mitigating this problem. It was this discovery that eventually opened a new era of essential FAs and brought FAs into the main frame of research. Figure 1 provides a glimpse of the early significant events in the history of FA research, highlighting the various milestones (setbacks and progress) in the history of development of FAs [2]. Since then, FAs have been the subject of significant research and are now a very well explored area.

The clouds of skepticism over role of FAs have sufficiently nullified and the new findings have resulted in a society that understands diseases very well and can relate more aptly to the FA connection to these diseases. In fact, knowledge about the role of particular FAs and how they exert their effect has expanded greatly over the past decade, so to effectively determine their benefit to health and nutrition. In nature, there is abundance of FAs, but they are never available in free form and are always found in the form of larger molecules called fat, which are energy dense (37 kJ or 9 kcal/g). Presently, there is much better understanding of how fats and FA are metabolized and used in the body. Much is known about how FAs alter cell membrane function, control gene transcription and expression, and interact with each other. Fats and FAs should now be considered key nutrients that affect early growth and development and nutrition-related chronic diseases later in life. Recent

This work was supported by the National Institute of Pharmaceutical Education and Research (NIPER) Ahmedabad. The Ministry of Chemicals and Fertilizers provided funding. The Department of Science and Technology provided funding to the authors in the form of INSPIRE faculty award (grant no. LSBM -13; SERB EMR/2016/007966). The authors have no conflicts of interest to declare.

* Corresponding author. Tel.: +91 79 667 45555; Fax: 91 79 667 45560.
E-mail address: mtbitat@gmail.com (M. Misra).

https://doi.org/10.1016/j.nut.2019.03.008
0899-9007/



1930	1930-1935	1930-1955	1955-1965	1968	1970-1978	
G. Burr and G. Hidtch published papers on abnormalities in rats due to absence of linoleic acid and are able to bring the fatty acids on global platform	G. Burr whose findings were based on preliminary analysis further garnered support when Hume et al. and Andereson published a paper regarding importance of fatty acids in diet	DHA structure was determined by Hammond and Lundberg. -Fred Synder and Biran found out that brain and synaptic membrane majorly comprised of phospholipids called DHA, which also has a fatty acid-like structure	Skeptism persists despite many research papers, confirming the essentiality of fatty acids	Parenteral nutritional solutions given to adult male rats were deficient of fatty acids and patients developed skin rashes. The rash disappeared after administration of soyabean emulsion containing 86 g linoleic acid	Infants being treated with parenteral nutrition suffered rashes and other abnormalities when administered with Intralipid®, all these rashes and abnormalities were resolved	Ouerberg, Bang, Sinclair reported decreased incidence of cardiac diseases in Eskiomos consuming large amounts of fatty acids like DHA & EPA. Clinical studies done to prove that fatty acids play an important role in cognition and vision

Fig. 1. The figure shows how the research on FA has progressed step by step since the early 19th century. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid.

research on ω-3 and ω-6 FAs as essential nutrients and also as part of the overall fat supply indicates the correlation between the prevalence and severity of cardiovascular disease (CVD), diabetes, cancer, and age-related functional decline. Dietary fats provide the medium for the absorption of fat-soluble vitamins; are a primary contributor to the palatability of food; and are crucial to proper development and survival during the early stages of life-embryonic development and early growth after birth through infancy and childhood.

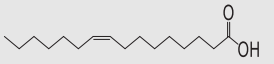
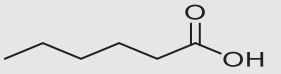
FAs have been shown to exist everywhere and in every organ of the human body, including membranes of all cellular components. The importance of FAs can be understood by the amount of research papers published and the data available on FAs among the scientific community. They have been extensively used in formulations to solve a variety of problems related to the human body. Additionally, FAs are used as potential antioxidants, anticonvulsants, and anti-cancer agents, as well as for treatment of several topical diseases. FAs are useful in the growth of human neurons, especially in early brain development and for neuroprotection to modulate gene expression and helpful for prostaglandin and many other required entities of the human body [3]. They also are a great source of alternative energy for the body.

This review focused on how FAs have contributed both as a therapeutic auxiliary and as a formulation aid. More specifically, we emphasized how FAs have changed the understanding of several biological processes such as permeation and diffusion and how they have influenced the delivery of an active agent, especially when administered via topical, oral, parenteral, ocular, or intranasal routes, to achieve brain targeting. The role of diet in fulfilling the body requirements of essential FAs (essential FAs are those that cannot be synthesized by the body but are obtained via diet) is also discussed. Additionally, we have elaborated on the role of omega FAs, touted as the emperor's pill, for treatment of diseases like multiple sclerosis, CVD, and cancer. With this review, our aim was to reduce the gap between the claims and scientific evidence and highlight the extensive research done in the area of FAs.

Connecting the dots: Influence of FA structure and their pharmacologic effect

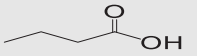
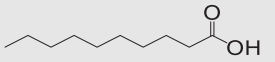
The way FAs act have been hypothesized to have some prerequisites, such as the degree of unsaturation, structural flexibility, hydrocarbon chain length, position of the double bond, and *cis-trans* conformation. According to their chain length and degree of unsaturation, FAs have been classified into short-, medium-, and long-chain FAs. Table 1 shows a list of different saturated (SFAs) and unsaturated FAs, their sources, and the therapeutic activity reported. These structural features of FAs are important in order to elicit the desired effects. On the basis of these structural features, FAs elicit different cellular functions such as triggering the anti-inflammatory pathway, thereby altering membrane fluidity, interaction with ion-gated channels, and conductivity and stability of

Table 1
FA: classification, structure, source and potential uses

Name	No. of carbon atoms	No. of double bonds	Symbol	Sources of FA	Potential and already established uses	GRAS and IIG listings of various FA	Structure
Palmitic acid [79,80]	16	–	16:0	Fresh red and white meat-poor source, Yolk-Richest source	Enhances effect of anti-viral therapy Palmitate ester has been used as a long-acting release carrier medium in the approved formulation of Invega Trinza which has been used for 3-mo antipsychotic therapy [81]	Palmitic acid – IV suspension, injection – 0.001% maximum potency per dose Palmitic acid –Tablet – 6 mg maximum potency per dose	
Stearic acid [79,80]	18	–	18:0	Sunflower oil (richest source) Lard and suet (highest animal sources)	Gives protection against oxidative stress. Stearic acid, has been studied as friction modifiers reducing the friction and wear of the nanoparticles [82] Stearic acid can be used as a hair growth-promoting agent [83]	Stearic acid is GRAS and IIG listed. IIG listing – Tablet (buccal) 5 mg Tablet (sublingual) 6 mg Capsule 52 mg Pellet 0.2 mg Powder for suspension 1203/5mL	
Caproic acid [79,80]	6	–	10:0	Animal fats like butter, cheddar cheese, and coconut oil	Caproic acid has been used to enhance gene delivery without affecting biocompatibility [84] It has been used to deliver protein and peptides. Example: enhancement in transdermal delivery of phenylalanyl-glycine was done by chemical modification via caproic acid, which enhanced its stability inside the skin [85]	*	
Lauric acid [79,80]	12	–	12.0	Coconut oil and palm kernel oil	Lauric acid has been used in the prevention of cardiovascular diseases as it promotes HDL and decreases the total/HDL cholesterol ratio, which results in a decrease in risk for cardiovascular diseases [86] Lauric acid helps in treating the inflammatory acne vulgaris by exerting antimicrobial activity [87]	*	
Myristic acid [79,80]	14	–	14:0	Coconut oil (richest source) and palm oil	Chronic administration of myristic acid significantly decreases hyperglycemia via decreasing the body weight and by decreasing insulin-responsive glucose levels and ultimately helps patients with type 2 diabetes [88] The appetitive responses in newborn humans seem to be driven by myristic acid [87]	Myristic acid is GRAS and IIG listed Capsule – extended release 215.3 mg maximum potency per dose. 2) GRAS listing safe	
Butyric acid [79,80]	4	–	4:0	Butter and parmigiana			

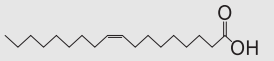
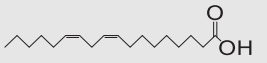
(*continued on next page*)

Table 1 (*Continued*)

Name	No. of carbon atoms	No. of double bonds	Symbol	Sources of FA	Potential and already established uses	GRAS and IIG listings of various FA	Structure
					Butyric acid (butanoic acid) is an important regulator of colonial microbial growth and an important regulator of apoptosis and anti – inflammatory activity. The butyric anion is easily absorbed by the enteric cells and provides huge source of energy to the human body [89] Butyric acid plays an important role in the regulation of the progesterone and estradiol (i.e., the hormonal signaling kicks off in action via the cAMP signaling pathway in the porcine granulosa cells) [90]	Butyric acid is GRAS and IIG listed as Transdermal- dosage form – Patch, controlled release	
Capric acid [79,80]	10	–	10:0	Cow butter, goat and coconut oil	The decanoic acid or capric acid has been used to inhibit the growth of *Candida albicans*, it has been reported to prove very effective in a combination against micellar growth of candida albicans as its fractionated inhibitory concentration index for 70% growth inhibition was calculated at 0.20, which is significant [91] Capric acid or decanoic acid may potentially influence the stimulation of the human osteoblast cells (MG63) as proven by Elakkiya venugopal et al. where they used the decanoic acid fractions of Wattaka volubilis leaves [92]	*	
Behenic acid [79,80]	22	–	22:0	The highest amount of FA source is present in peanut oil and peanut butter	Behenic acid has been reported to have a prognostic value for the detection of glial tumors (i.e. according to a report by Kaplan et al., the levels of other FA were decreased but the levels of behenic acid was found to be significantly higher in the glial tumors [93] The loss of oil is a day-to-day issue in high oil volume fat-structured foods as the *trans*-FA are absent so a stabilizer rich in behenic acids is added to these products to reduce oil migration and loss [94,95]	*	

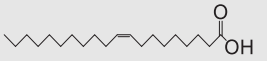
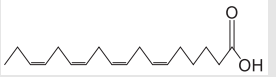
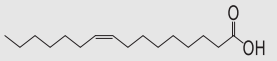
(*continued on next page*)

Table 1 (*Continued*)

Name	No. of carbon atoms	No. of double bonds	Symbol	Sources of FA	Potential and already established uses	GRAS and IIG listings of various FA	Structure
Unsaturated FA							
Oleic acid [79,80]	18	1	18:1n-9	Nut oil, olive oil, and canola oil	Oleic acid has been reported to behave as a tumoricidal agent when added to the nanoplex called LIMLET (liposome made lethal to cancer cells) and it was reported to be highly selective toward cancer cells in a concentration-dependent manner [96] Oleic acid has been used as a penetration enhancer on numerous occasions by many researchers and growth of the embryonic hippocampus AA protects brain cell damage by opening of the K^+ channels like TRAAK and TREK-1 [97]	*	
α-linolenic acid [79,80]	18	3	18:3n-3	Soybean oil and canola oil	The most easily available dietary source of the PUFAs which allows the conversion to DHA by the animals' liver and hence ALA acts as a precursor for making DHA	*	
EPA [79,80]	20	5	20:5n-3	Cod liver oil and many fish oils	EPA, which is a very important ω-3 FA and is called the emperor's pill, is used to treat many diseases like cardiovascular and neuronal diseases and is used as an auxiliary in many formulations acting as a penetration enhancer, stabilizer, etc.	*	
Docosapentaenoic acid (DPA)	22	5	22:5n-3	Cod liver oil and many fish oils	High plasma levels of are associated with reduced risk for bleeding post-cardiac surgery [99]	*	
DHA	22	6	22:6n-3	Cod liver oil and many fish oils	DHA supplementation increases and improves memory and cognitive power [100]	*	
Linoleic acid	18	2	18:2n-6	Fats of walnut, peanut, livestock fed diet particularly rich in FA	Linoleic acid is used in the production of prostaglandins. Linoleic acid is a major constituent of the acyl glycosyl ceramides that plays an important role in maintaining water permeability barrier of skin. Linoleic acid aids in production of arachidonic acid which is a major precursor of eicosanoids production [101]	*	
ARA [79,80]	20	4	20:4n-6	Meat, eggs and fish	ARAs are postulated to rebuild the damage done to the neural	ARA [79,80]	

(*continued on next page*)

Table 1 (*Continued*)

Name	No. of carbon atoms	No. of double bonds	Symbol	Sources of FA	Potential and already established uses	GRAS and IIG listings of various FA	Structure
					cells as it is deemed that its absence leads to vascular fragility and insufficiency in the newborn infants [102]. ARA has also been said to promote neurite growth of the embryonic hippocampus. ARA protects brain cells damage by opening of the K^+ channels like TRAAK and TREK-1 [98]		
Gadoleic acid (9-Eicosenoic acid [79,80]	20	1	20:1n-1	It occurs as a glycerol ester in fish like cod-liver oil and rapeseed	Adhesives and chemical agents. Surface active agents. Agricultural pesticides. Lubricant and lubricant additives.	*	
Erucic acid [79,80]	22	1	22:1n-9	1) Erucic acid occurs as glycerol ester in the seed fats of rapeseed, mustard, broccoli, kale, wallflower, it is also isolated form seeds of Raphnus sativus.	Erucic acid plays a role in causing oxidative burst in human polymorpho-nuclear leukocytes [103] Erucic acid can play a potential role in treating patients with diseases like X-linked adrenoleukodystrophy [104]	*	
Mead acid (5,8,11-eicosatrienoic acid) [79,80]	20	3	20:3n-9	Mead acid is found more in the cartilage and also in some fungi and generally produced by recombination	Mead acid can play a potential role in suppressing growth and metastasis of cancer via modulating the VEGF signaling especially in breast cancer [105] It also has the potential to modulate human allergic and inflammatory reactions, as it get converts to leukotrienes C3, D3, and 5-oxo-eicosatrienoic acid which is an analog of 5-oxo-eicosatetraenoic acid (a potent stimulator of human blood eosinophils and neutrophils) [106]	*	
Stearidonic acid [79,80]	18	4	18:4n-3	It is available as a glycerol ester in the borage leaf and black currant seed oil (example, ribes nigrum of the family Saxifragaceae) 2) Echium oil	Stearidonic acid may help in alleviating the inflammation by preventing the activation of the inflammatory NF-κB and MAPK pathways [107] It can play all the potential roles of EPA and the other LCPUFAs [107]	*	
Palmitoleic acid [5,79,80]	16	1	16:1n-7	They are met in the form of glycerol ester in marine source and vegetable seed oils. Example: Seed oils like Roureopsis obliquifoliata, Asclepis syriaca and Echuim oil	It is proposed to have antithrombotic effects that can help prevent stroke [108] It has been proposed to induce satiety and release the appetite-related hormones [109]	*	

(*continued on next page*)

Table 1 (*Continued*)

Name	No. of carbon atoms	No. of double bonds	Symbol	Sources of FA	Potential and already established uses	GRAS and IIG listings of various FA	Structure
Lauroleic acid (9-dodecanoic acid) [79,80]	12	1	12:1n-3	It is seen in the form of glycerol ester in animal fats present in milk and butter	It being uncommon in nature is not readily available and occurs only in small amounts but being an ω-3 FA it is hypothesized to show same effects as of other ω-3 FA like treating cancer, inflammation, and cardiovascular diseases [109]	*	
Vaccenic acid (11 octadecenoic acid) [79,80]	18	1	-	Found in form of glycerol ester in the fats of animals like ox and sheep Hydrogenated plant oil is found to be another important source of vaccenic acid.	Vaccenic acid administration for a chronic period of time may modulate the response in immunosuppressive conditions [109] Vaccenic acid has found to benefit models with dyslipidemia especially by lowering hypertriglyceridemia even though further studies are warranted to prove the effect on animals a preliminary investigation on rodents suggests the above possibility [109]	*	

*Data not availableALA, α-linolenic acid; ARA, arachidonic acids; cAMP, cyclic adenosine monophosphate; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GRAS, Generally Recognized as Safe; HDL, high-density lipoprotein; IIG, Independent Investigations Group; IV, intravenous; LC, long-chain; PUFA, polyunsaturated fatty acid; MAPK, mitogen-activated protein kinase; NF, nuclear factor; TRAAK, Twik-related acid-arachidonic activated K^+ channel; TREK, TWIK1-related K^+; VEGF, vascular endothelial growth receptor

formulations, which are discussed in detail in the proceeding sections.

The FA have been extensively well documented and classified in a stereotyped manner via the publication of a great deal of research papers since the 1990s with fewer being added in the recent past, although the importance of many FA has been lobbied for only in recent times.

Effect of the FA unsaturation and structural modifications on the membrane fluidity

Polyunsaturated fatty acids (PUFAs) have been established in treating various diseases such as Alzheimer's disease, via different pathways, one of which is their ability to trigger the anti-inflammatory pathway in the cell membrane, thereby altering cell membrane fluidity [4]. Research published by Yang et al. [5] discussed this hypothesis and related membrane fluidity and amyloid precursor protein (APPα) processing with the degree of unsaturation of FAs. Yang et al. [5] investigated the effects of FAs with different unsaturations on the membrane fluidity (from 0-6 double bonds). The FAs selected for the study were stearic acid (SA; 18:0), oleic acid (OA; 18:1), linoleic acid (LA; 18:2), α-linolenic (ALA; 18:3), arachidonic acid (ARA; 20:4), eicosapentaenoic acid (EPA; 20:5), and docosahexaenoic acid (DHA; 22:6). It has been hypothesized that these FAs are effective in altering membrane fluidity. To study this hypothesis, different concentrations of FAs were prepared (including DHA and EPA) and studied for their effect on membrane fluidity using the human neuroblastoma SH-SY5Y cell line. The purpose of the study was to see how different FAs would affect the membrane fluidity and whether the structural differences in FAs have a role in altering the membrane fluidity. To test whether the position of double bonds plays a role in membrane fluidity, the SH-SY5Y cells were exposed to *cis*-2-eicosenoic acid, *cis*-5, 8, 11-eicosatrienioc acids, which have the same number of double bonds as OA and ALA acid. Yang et al. [5], after extensive studies, concluded that the FAS having less than four double bonds, namely the SA, OA, LA, and ALA, have no significant effect on membrane fluidity of the cell membrane. The study further revealed that the position of the double bond does not influence membrane fluidity and only the number of double bonds in a surfactant (FA) has a significant effect on the fluidity of cell membrane [5]. In other research published in 1989 [6], regarding the effect of FA unsaturation on membrane fluidity, the authors used male guinea pigs and divided them into two groups. The first group was fed a complete semi-synthetic diet according to Reid and Briggs. The second group, a fat-deficient group, was given the same diet with glucose instead of corn oil. The lipid-deficient diet resulted in the substitution of the OAs for LAs (i.e., a redistribution of the unsaturation was observed without an altered ratio of the phospholipids to cholesterol molar ratio or polar head group composition ratio). The link between the effects of unsaturation on membrane mobility was evaluated by use of a fluorescent pyrene probe, which helps us understand translational mobility. The efficiency with which pyrene excimer formation takes place is indicative of the rates of lateral diffusion in the endoplasmic reticulum. The I_e:I_m ratio is the ratio of the pyrene excimer divided by the fluorescence intensity of the pyrene monomer (I_e:I_m), which was calculated from the fluorescence intensities at 472 and 392 nm, respectively. The I_e:I_m ratio shows the direct relationship between degree of unsaturation and translational mobility. A lower I_e:I_m ratio suggests decreased translational mobility, which in turn is caused by decreased levels of membrane unsaturation. The higher ratio suggests an increased translational mobility due to increases in the level of unsaturation.

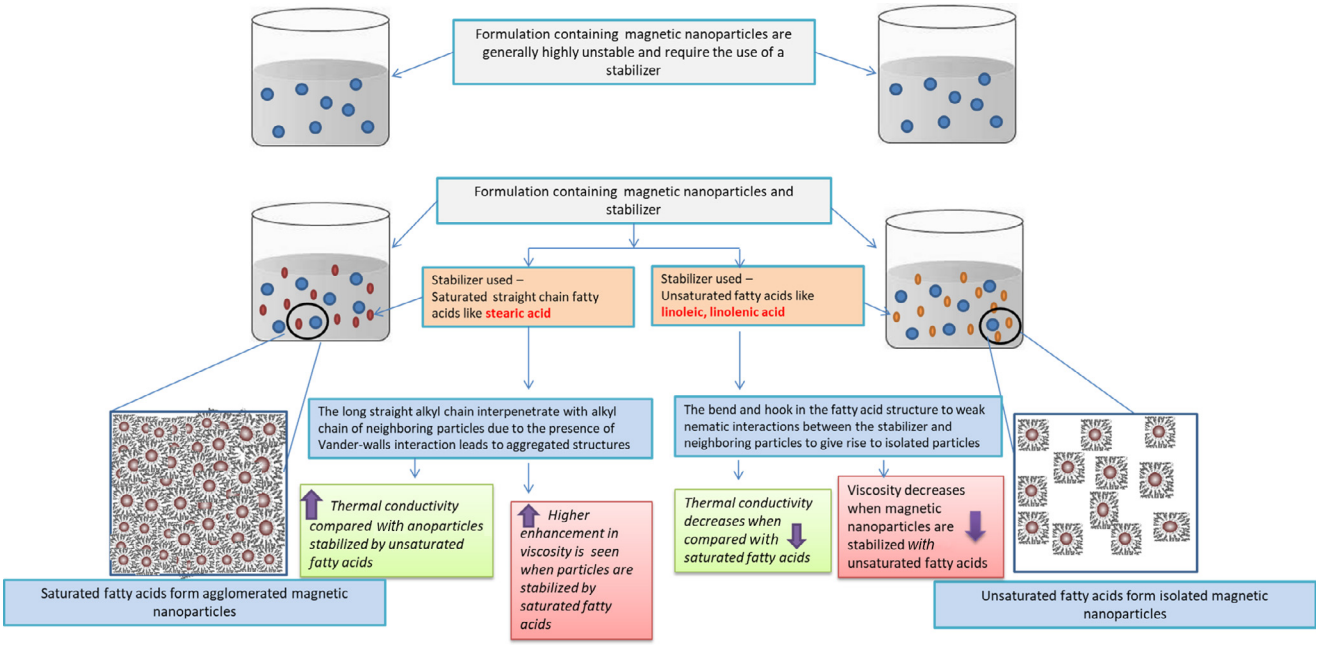


Fig. 2. Effect of FA unsaturation on stabilization, viscosity, and conductivity of magnetic nanoparticles. Changing the stabilizer (saturated or unsaturated) effects the formulation stability. The use of saturated FAs as surfactants formed aggregated magnetic nanoparticles, whereas use of unsaturated FA gave rise to single isolated magnetic nanoparticles. The saturated FA increase the thermal conductivity and viscosity as compared with the unsaturated FA. FA, fatty acid.

Castuma et al. [6] showed that the I_e:I_m ratio value is lower for the fat-deficient microsome than for the normal microsome, indicating that the level of unsaturation decreased in the fat-deficient microsome when OA was substituted in place of LA. OA is a monounsaturated fatty acid (MUFA), whereas LA is a PUFA, and in fat-deficient microsomes it is hypothesized that LA is substituted with OA. The introduction of a *cis* double bond has led to the disruption of membrane and hence the fluidity of the cell membrane was increased [6]. This was attributed to the breaking of the new *cis* double bond into the tightly packed *trans* bonds of the saturated fatty acyl chain, thus increasing the cross-sectional area and eventually the so-called fluidity [7].

Effect of nature and structural modification of FA on conductivity, viscosity, and stability of magnetic nanoparticles

The FA unsaturation also has been important in the stabilization of magnetic nanoparticles, influencing both their conductivity and their viscosity. They have been widely used for stabilizing the magnetic nanoparticles in aqueous as well as non-aqueous medium; numerous studies have been conducted to understand how the unsaturation and saturation of Fatty acids (FA) will affect the viscosity and conductivity of magnetic nanoparticles. These studies evaluated whether the different bend, hook, and kink confirmations of LA, ALA, and OA, respectively, have any influence on stabilization of the magnetic nanoparticles. It was found that the nanofluids with SFA concentrations showed more viscosity than the fluids with unsaturated FA concentrations [8]. The nature of the surfactant plays a role in the thermal conductivity, as explained by two researchers independently. Philip et al. [9] reported preparation of ferric sulphate magnetic nanoparticles using OA and capric acid as stabilizers. The results showed that unsaturated/branched surfactants like OA have a tendency to form slightly assembled particles; whereas results from a study conducted by Altan et al. [10] revealed single-/linear-chain surfactants form more aggregated structures. The viscosity, solvation, and conductivity of the magnetic nanoparticles are interrelated and affected by the choice of FA as a surfactant. The schematic diagram shown in Figure 2 describes the interrelation between viscosity, solvation, and conductivity to the unsaturation of the FA used.

Different types of channels and how the type and structure of FA influence the activity of the channels

There are numerous channels and pathways through which many types of signals are generated, which ultimately leads to different therapeutic effects. To elicit their potential effect, such as hypertension and other reported effects of FA, FAs must interact with the receptors and channels present on the cell to start the cascading cellular events [11].

Yazdi et al. [7] investigated the interaction of various types of SFAs and unsaturated FA to analyze whether there was any difference in the way unsaturated and SFAs interacted with the various channels present on the cell membrane. Such studies further add to the understanding of how structural differences between FAs influence activity at the cellular level. The effect of PUFAs on the ion channels has been investigated. PUFAs are the largest class of signal-transducing proteins and play a major role in conducting nerve impulses, aiding in muscle contraction and hormonal control. The PUFAs, which are a part of the cell membrane, are reported to activate these channels via shifting the voltage dependence of the K_v (K^+) channels. The channel is divided into six *trans* membrane helices, of which the first four (S1–S4) make up the voltage sensor domain on which mainly the PUFAs and SFAs act [12]. The activation of these then activates the central conducting pore comprising the (S5–S6) *trans* membrane helices. The authors have performed various studies to determine the effects of unsaturation, which affect the structural dynamics and eventually the opening and closing of the shaker voltage-gated potassium channels. They simulated the lone PUFA (DHA) and lone SFA (DA) molecules in two separate 1–palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane patches. The PUFAs and SFAs

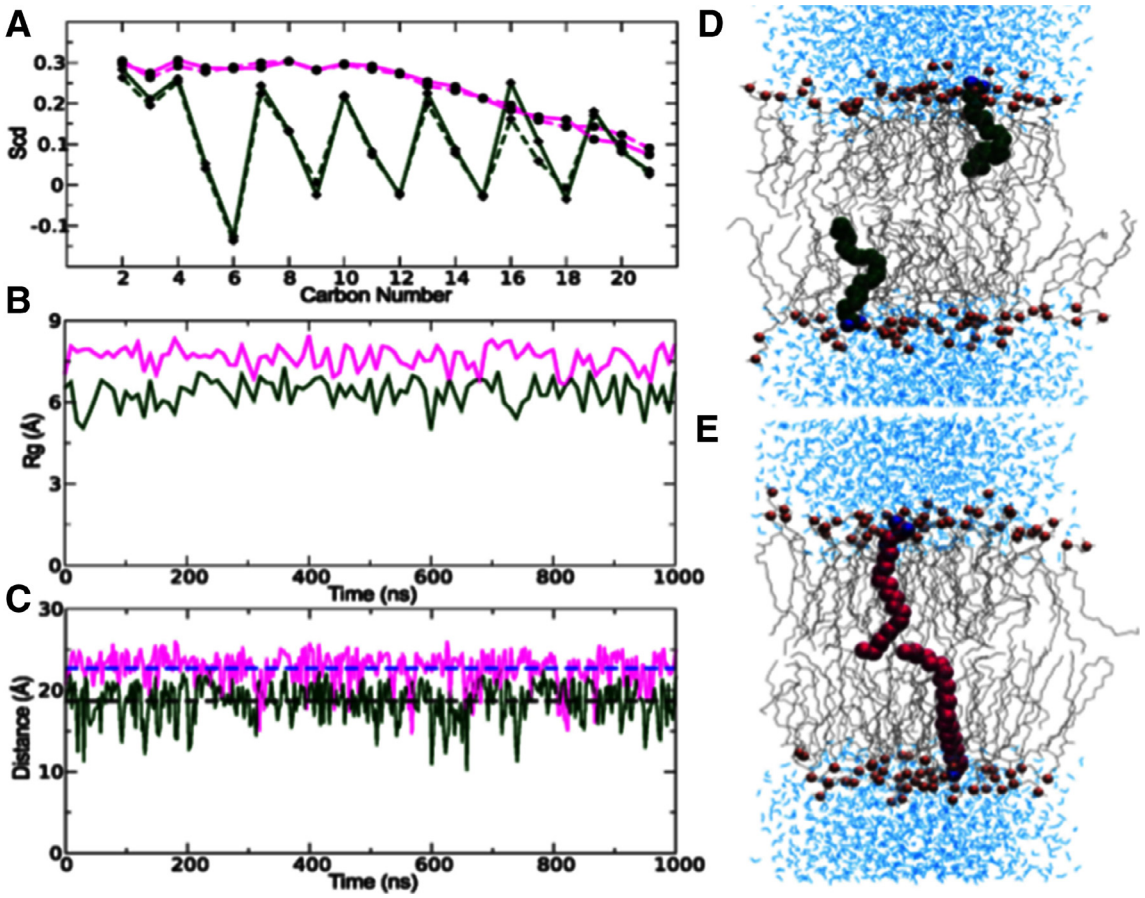


Fig. 3. Structural flexibility in lipid partitioned PUFA and SFA. (**A**) Deuterium order parameters of PUFA (green) and SFA (purple) carbon chains. (**B**) The radius of gyration of PUFA (green) and SFA (purple). (**C**) Distances between the head group oxygen and last carbon chain in the PUFA (bottle green) and SFA (purple) chains. Dotted lines denote the overall average distance for the PUFA (black) and the SFA (blue), respectively. Representative structures from 1 μs simulations of PUFA (**D**) and SFA (**E**) are shown embedded in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine bilayers. PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid (Adapted from [7].)

had distinct interactions with the channels, mainly owing to the difference in the conformational flexibility of PUFAs and SFAs. The SFAs are more rigid and had straight chain conformation, which does not allow them to fit inside the binding pocket, whereas the PUFAs, owing to their unsaturation (kinks and bends), fit easily inside the binding pocket. The tail flexibility of PUFAs is confirmed by many studies, namely the rhodopsin studies, quantum mechanical calculations, and nuclear magnetic resonance studies [13,14]. The radius of gyration, which refers to the distance from the axis at which the mass of a body may be assumed to be concentrated, was observed to be smaller for the PUFAs than the SFAs. This smaller radius of gyration further indicates the conformational flexibility of PUFAs over SFAs. The distance between the carboxyl head group oxygen and the final methyl carbon in the tail (O-C22 distance) was shorter in PUFAs by 5Å than the SFAs. The interactional difference between the PUFAs and the shaker voltage-gated potassium channels was studied in both open and closed state of the channels. There were only minor differences such as the protein residue contacting PUFA head groups, as seen in the S3, S4 helices in open state, whereas for the SFAs these were seen prominently in the S1–S2 helices. Half of the contact points between the protein SFA head and tail groups occurred only once and hence were unique [7,12]. Figure 3 depicts the structural flexibility of the PUFAs over the SFAs.

PUFAs have been reported to act as a therapeutic aid to treat diseases related to the heart (atherosclerosis, coronary heart disease), brain (Alzheimer's), and other diseases such as cancer and diabetes [15]. To show the above-mentioned health benefits, PUFAs interact with the receptors present on the smooth blood vessels in heart. Tian et al. [16] prepared numerous PUFA derivatives to understand this interaction at the cellular level. They have investigated these derivatives to determine if the length of the carbon chain and the number and position of the double bond play any significant role in PUFAs' interaction with the ion-gated channels. They have researched the structural prerequisites required for the large-conductance Ca^{2+} and voltage-gated Slo1 BK channel activation and the hypotensive action provided by the PUFAs for the organism. Through various studies, they concluded that the PUFA tail length was a significant contributor to the affinity characteristics required for the interaction between the channel and the PUFA. This conclusion was backed by the EC_{50} values, which were markedly different for 18 carbon chains and 22 carbon tails, the 18-carbon tail had an EC_{50} value of 7 μm as compared with only 0.5 μm for the 22 carbon chains, which was almost 14 times compared with DHA. This significant difference was attributed to the FA off-kinetics of the 18-carbon tail PUFA [16].

Another important parameter is the conformation, which is required for a stable interaction of PUFA, with the channel, wherein the double bond and the kinks contributed toward the rigidity of the structure. The Z double bond near the central point of the tail implies a curved and not so prominently compact structure is required to activate the channel in a stable manner (i.e., for a certain duration of time). The prima facie research states that without the Z double bond, the PUFA tail is lacking rigidity and

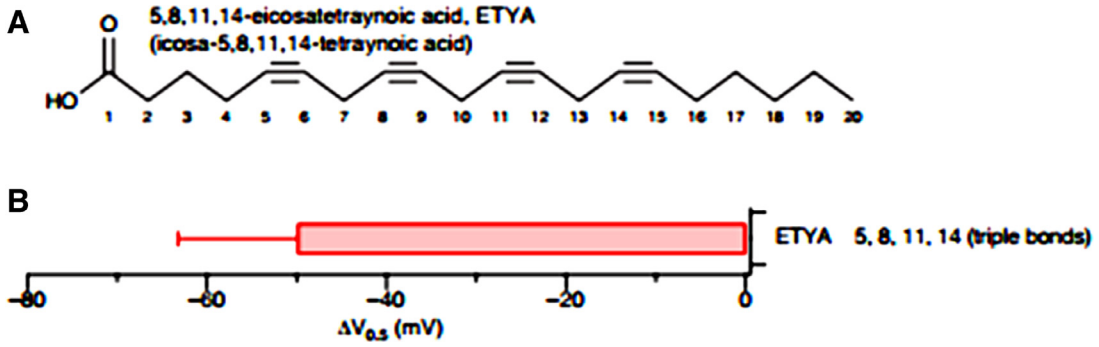


Fig. 4. (**A**) ETYA (5,8,11,14- eicosatetraenoic acid) structure (**B**) $V_{0.5}$ change in Slo1+β1 by ETYA (3 q M) (Adapted from [16].)

seems flexible, free, and unable to occupy the interaction pocket on the channel for a period that the channel will stay active for a considerable period of time. The effect of the addition of a triple bond was evaluated in order to identify the importance of the tail bond rigidity and to test the above-mentioned hypothesis that a bond in PUFA tail helps the molecule to occupy the binding site for a considerable period of time so that activity is seen. They generated a molecule called ETYA (5,8,11,14- eicosatetraenoic acid), comprising triple bonds in the structure, and observed that this structure was able to interact with the channel, altering the $V_{0.5}$ value considerably. Figure 4 shows the importance of the triple bond for rigidity of the tail and shifting potential $V_{0.5}$.

The effect of the position of the double bond was evaluated and it was determined that docosatetraenoic acid, which does not have any double bonds at the 4,7,10 position, shows significantly diminished ability to alter the potential of $V_{0.5}$ (indicates the potential of the channel, as the value drifts more toward the negative side, the better the interaction with the channel). The absence of double bond at the 19 position leads to an almost similar shift in the potential ($V_{0.5}$). Similarly the absence of double bond at the 8, 11 position in PUFAs leads to huge alteration in the $V_{0.5}$ value [16]. Figure 5 highlights the importance of the position of the double bond within the PUFA chain.

Application of FAs in topical formulations

Rational approaches to the development of topical formulations necessitate the study of physical and chemical properties of the drug and vehicle components added. This is essential to facilitate the passage of the drug across various cellular barriers, which is a formulation that it will encounter until it reaches the site of action. The presence of epithelial tight junctions, and rapid drainage of the instilled drug solution by tears from the pre-corneal area of eye, results in the permeation of <5% of the applied dose of a drug. Similarly, in the presence of dead, flattened, keratin-rich cells, the corneocytes surrounded by a complex mixture of intercellular lipids

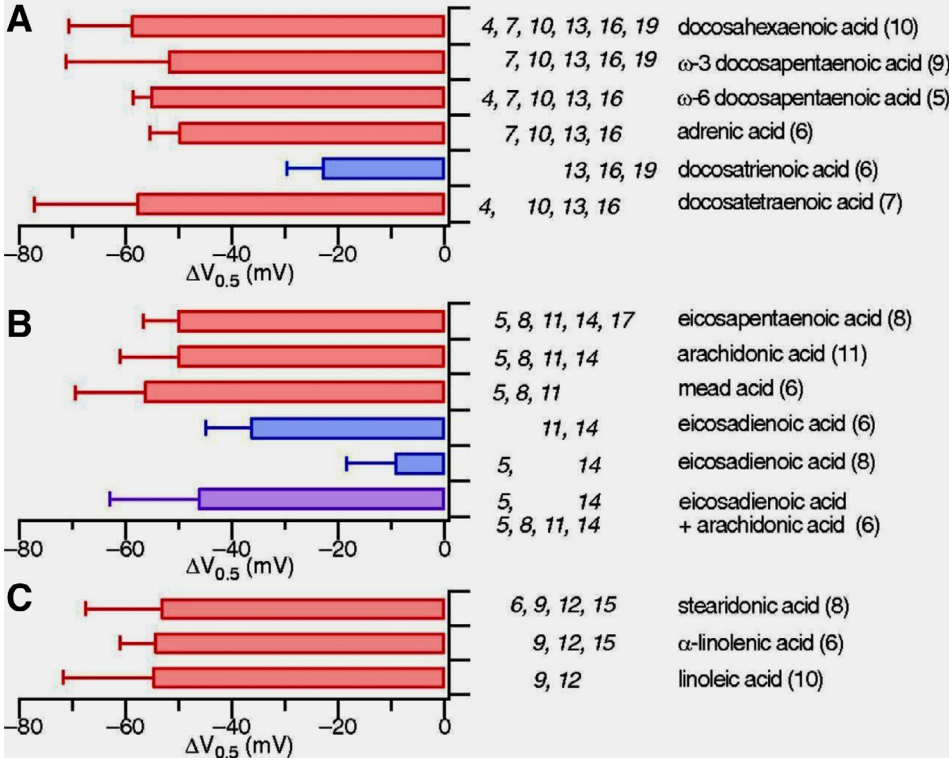


Fig. 5. Contributions of PUFA double bonds (**A**) Changes in $V_{0.5}$ by 3 μM PUFA with 22-carbon tail groups; (**B**) Changes in $V_{0.5}$ by 3 μM PUFA with 20-carbon tail groups; (C) Changes in $V_{0.5}$ by 30 μM PUFA with 18-carbon tail groups. The numbers indicate the double bond positions. PUFA, polyunsaturated fatty acid. (Adapted from [16].)

pose a formidable barrier for delivery of the drug to skin. In following section, we discuss how FAs have been used to overcome these barriers.

Fatty acids: Tackling the ocular barrier for drug delivery

The ocular delivery has always been a challenge; FAs have been extensively used to solve many of these formulation and therapeutic hurdles. Ocular drug delivery to the posterior region of the eye has been a task for all scientists working in this domain and a considerable amount of progress has been made in this area. The limitations of the various routes employed to deliver the drugs to the posterior segment of eye have been discussed extensively and a topical route needs to be researched more as this route has the highest patient compliance and is the most sought-after route [17–19]. FAs can help to fulfill this objective by serving the dual purpose of drug delivery and as a therapeutically active agent in treating the diseases of the posterior segment of the eye as well as other parts [20].

FAs have wide-ranging applications and can be used as virucidal [21] or antimicrobial/antibacterial [22,23] agents to treat dry eye syndrome [24] and age-related macular degeneration [25], and at the same time in drug delivery [26] to increase the amount of drug reaching the posterior segment of the eye, which has been an issue for many researchers working on ocular drug delivery. The FAs are unique in nature as they do not harm the host cells and are able to exert sound antibacterial and virucidal activity even when used at a significant concentration. They do not develop resistance like other agents such as antibiotics, which fail to show any antimicrobial and virucidal effects once the bacteria and viruses garner resistance [27].

In a research study conducted by Churchward et al. [28] who used an FA comprising formulation to prevent ophthalmia neonatorum, a disease caused by bacterium *Neisseria gonorrhoea*, which infects the newborn's eyes as the child passes the birth canal. The bacterium causes inflammation to the corneal tissue with a yellowish discharge from the closed eye, which may further lead to blindness. The third generation of cephalosporins and tetracyclines emerged as excellent prophylaxis treatment around the 1950s but their use faded quickly due to the emergence of multidrug resistant *N. gonorrhoea* species. The authors screened ~37 FAs for their antigonoccocal activity and were able to find lauric acid, tridecanoic acid, myristoleic acid, palmitoleic acid, linolenic acid, and monocaprin acid significantly active. Seven FAs were found to be active and were further screened using several tests. These seven FAs were initially selected based on their anti-gonococcal activity, which was quite substantial. Table 2 the activity profile of the seven FAs.

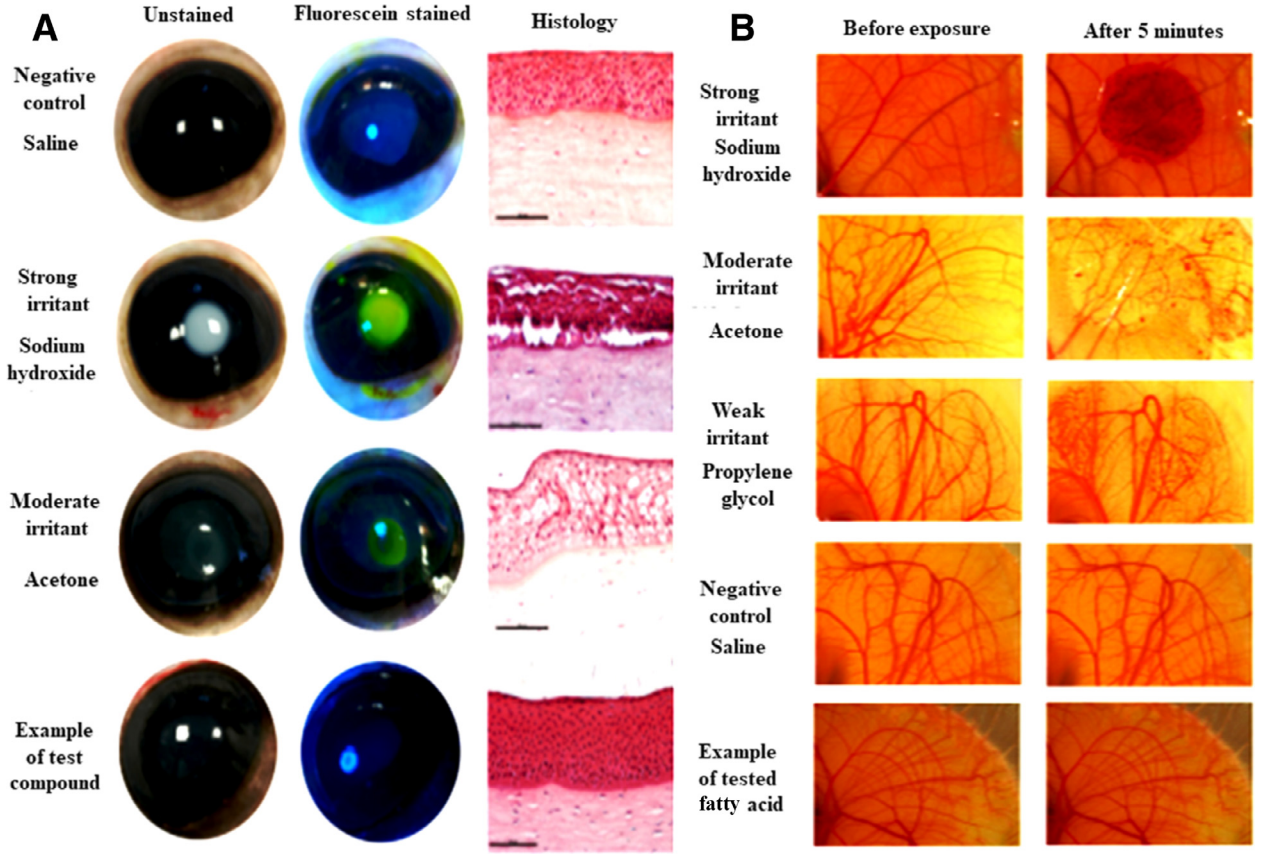


Fig. 6. **(A)** Unstained and fluorescein-stained bovine eyes exposed to components used in the BCOP test. Positive controls for irritation (sodium hydroxide and acetone) indicate the opacity of the cornea, and staining reveals changes in permeability; Histology micrographs (right) show hematoxylin and eosin (H & E)-stained sections of the epithelial layer of the cornea. Positive controls show extensive cytopathic damage to the epithelial cell layer, with complete detachment in the case of sodium hydroxide. The negative control with non-irritating saline solution and all of the candidate organic acids showed no opacity of the cornea, no notable staining with fluorescein, and no damage to the tissues revealed by histology. The scale shown on histology images is equivalent to 100 μm. **(B)** Photographs from HET-CAM test. Eggs were grown for 9 d, and then the tested components were spotted onto the chorioallantoic membrane for 5 min. The top six images show positive controls where the irritants caused hyperemia and clotting at the site of application upon comparison before application (left) and 5 min after application (right). These changes were not seen in the negative control when saline solution was applied or in the tested candidates, where application did not cause a reaction. Application of lauric acid (12:0) is shown in the bottom pair of images as an example for all candidates. BCOP, bovine corneal opacity and permeability; HET-CAM, hen egg test–chorioallantoic membrane (Adapted from [28].)

Table 2
Activity profile of FA tested against bacteria *Neisseria gonorrhoea* [28]

Chemical class	Organic FA	Growth inhibition*	Log reduction at 1 mM†	MBC (mM)‡
Saturated FA	Lauric acid	Yes	Yes >6	0.75
	Tridecanoic acid	Yes	Yes >6	0.75
Monounsaturated FA	Myristoleic acid	Yes	Yes >6	0.5
	Palmitoleic acid	Yes	Yes >6	0.5
Polyunsaturated FA	Linoleic acid	Yes	Yes >6	0.5
Monoglycerides	Monocaprin	Yes	Yes >6	0.5
Ricinoleic acid	Ricinoleic acid	Yes	Yes >6	–
FA sodium salt	Sodium myristate	Yes	Yes >6	0.75
	Sodium dodecanoate	Yes	Yes >6	

FA, fatty acid; minimum bactericidal concentration (MBC); NT, not tested.
*The bacteria did not grow in GC agar media containing a 1 mM concentration of the organic FA candidate under test.
†The data represents the calculated log reduction in level of viable bacteria after exposing the bacteria to the organic.
‡Data represent the calculated log reduction in the level of viable bacteria after exposure to a 1 mM concentration of the candidate for 2 min.

The seven FAs were further screened on the basis of cytotoxicity assessment by performing the bovine corneal opacity and permeability (BCOP) assay and hen egg test–chorioallantoic membrane (HET-CAM) assay. The BCOP assay addresses the corneal irritation potential and HET-CAM test is a gold standard model to mimic conjunctiva. The seven FAs successfully cleared all the cytotoxicity tests [29]. The BCOP and HET-CAM test further validated the safety hypothesis of FAs. Figure 6 shows the outcomes of the two safety assays performed [28]. This research further strengthens the concept that FAs are relatively safe, cheap, and effective drug delivery and therapeutic agents.

Posterior eye diseases, as mentioned earlier, are a challenge, and current routes for drug delivery to that area of the eye require use of repeated injections, causing pain and eventually leading to non-compliance [30,31]. This has led to exploration of novel penetration enhancers to deliver drugs via topical route. FAs have been explored by scientists as penetration enhancers for topical drug delivery in the past decade. Raval et al. [32] studied the role of butter oil, which comprises numerous FAs, as a novel, safe penetration enhancer for topical (ocular) drug delivery. Microemulsion as a dosage form for drug delivery was used and butter oil was added to enhance drug delivery to the posterior segment of eye. Triamcinolone acetonide (TA) was used in this study as a model drug due to its low ocular bioavailability; butter oil was added to the microemulsion and was compared with the already established posterior ocular drug delivery agent called chitosan [32]. This comparison with chitosan was done on the grounds of its traditional use as a penetration enhancer in ocular drug delivery due to the positive charge on the surface of the polymer, which allows it to adhere with the negative charge present on the surface of the eye.

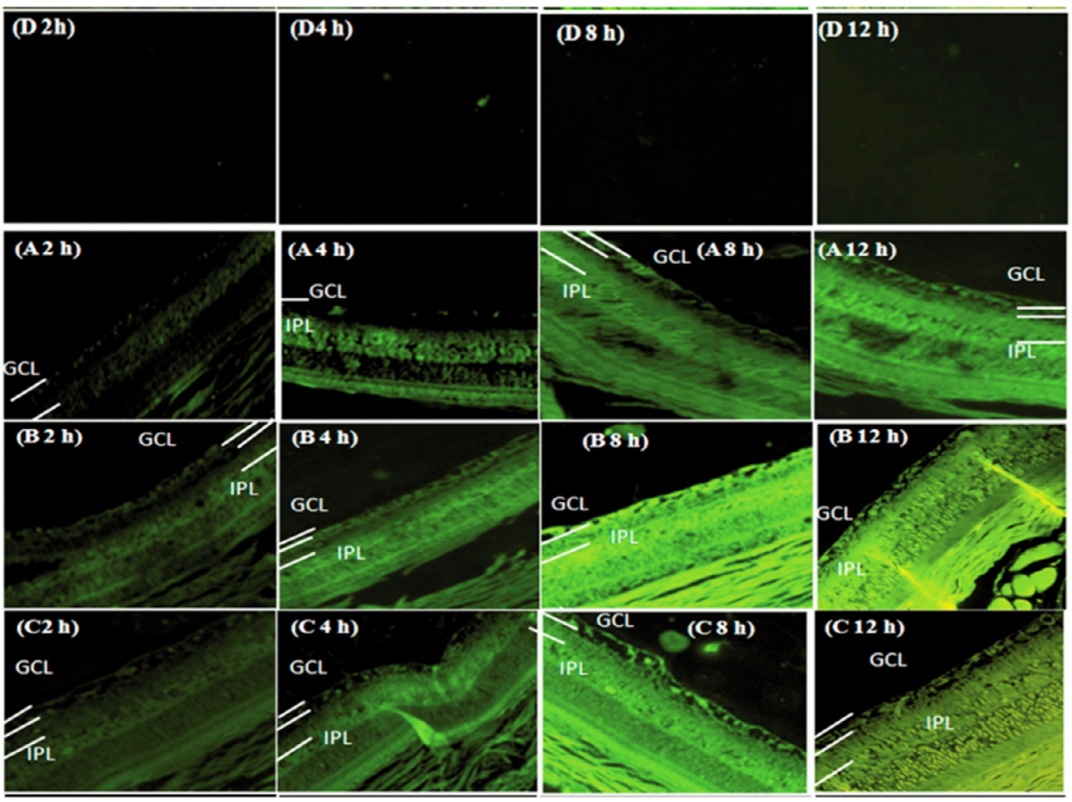


Fig. 7. Images of the posterior segment of the eye on treatment with coumarin-6 which acts as a fluorescent agent. (**A**) Coumarin-6 loaded microemulsion; (**B**) Coumarin-6 butter oil-loaded microemulsion; (**C**) Coumarin-6 chitosan-loaded microemulsion; (**D**) Contralateral eye versus control for a period of 12 h. (Adapted from [32].)

Previously, researchers showed chitosan to act differently than FA and its main role was to improve the retention time inside the eye, in order to allow the drug sufficient time to show its activity [33]. Probable mechanisms of penetration enhancement by FAs could be their interaction with the ocular lipids. These are known to alter membrane fluidity by quite a few purported mechanisms such as altering the melting temperature of the phospholipid bilayer, which causes the gel–fluid transition of the phospholipid bilayer enabling the entry of the drug via the tight junctions present in the eye, as reported by Calder et al. [34]. The unique composition of FAs present in butter oil also are hypothesized to contain DHA and various PUFAs, which make up most of the lipid component present inside the photoreceptors, which in turn fill up the retinal outer segment membranes. The use of FAs to treat many diseases of the eye such as age-related macular degeneration and dry eye syndrome, which affect the retina primarily is well explored by other researchers [35]. Raval et al. studied the efficacy of butter oil in penetrating to the posterior region of eye by using a fluorescent dye called coumarin-6. Figure 7 describes the ability of various formulations to reach the posterior segment of the eye. The images shown comprise the posterior eye sections, which are stained with coumarin-6 at different time intervals [32].

FAs have been studied for ophthalmic delivery and have successfully mitigated many challenges of drug delivery. The therapeutic effect of FAs comes from PUFA category consisting of DHA and EPA, which are reported to generate metabolites exhibiting anti-inflammatory and organelle (retinal, corneal) healing properties. These FAs are used to treat diseases like age-related macular degeneration, dry eye syndrome [36], and the like. The metabolites, namely resolvins and neuroprotectins, help to elicit the anti-inflammatory properties of the FAs, whereas the neuroprotectins D1 (NPD1) and other mediators help in tear film formation and give respite to patients with dry eye syndrome. The FAs bring notable modifications in parameters like the Ocular Surface Disease Index (OSDI), tear breakup time, and Schirmer's test, which signifies an improvement in overall eye functioning (Fig. 8) [37–39]. There are numerous formulations for eye ailments available the market that contain different types of FAs and are useful from a therapeutic point of view or as formulation additives [40].

Diverse role of FAs in topical formulation

FAs, since ancient times, have been used to treat a variety of skin and topical diseases. They have contributed immensely as a therapeutic aid and as an effective agent for enhancing the penetrability of drugs for a variety of diseases such as psoriasis, mucosal infection, and many other topical diseases. FAs in topical formulations have been used as an excipient, which helps to improve the overall stability of the formulation and at the same time providing a synergistic benefit to the overall activity of the formulation.

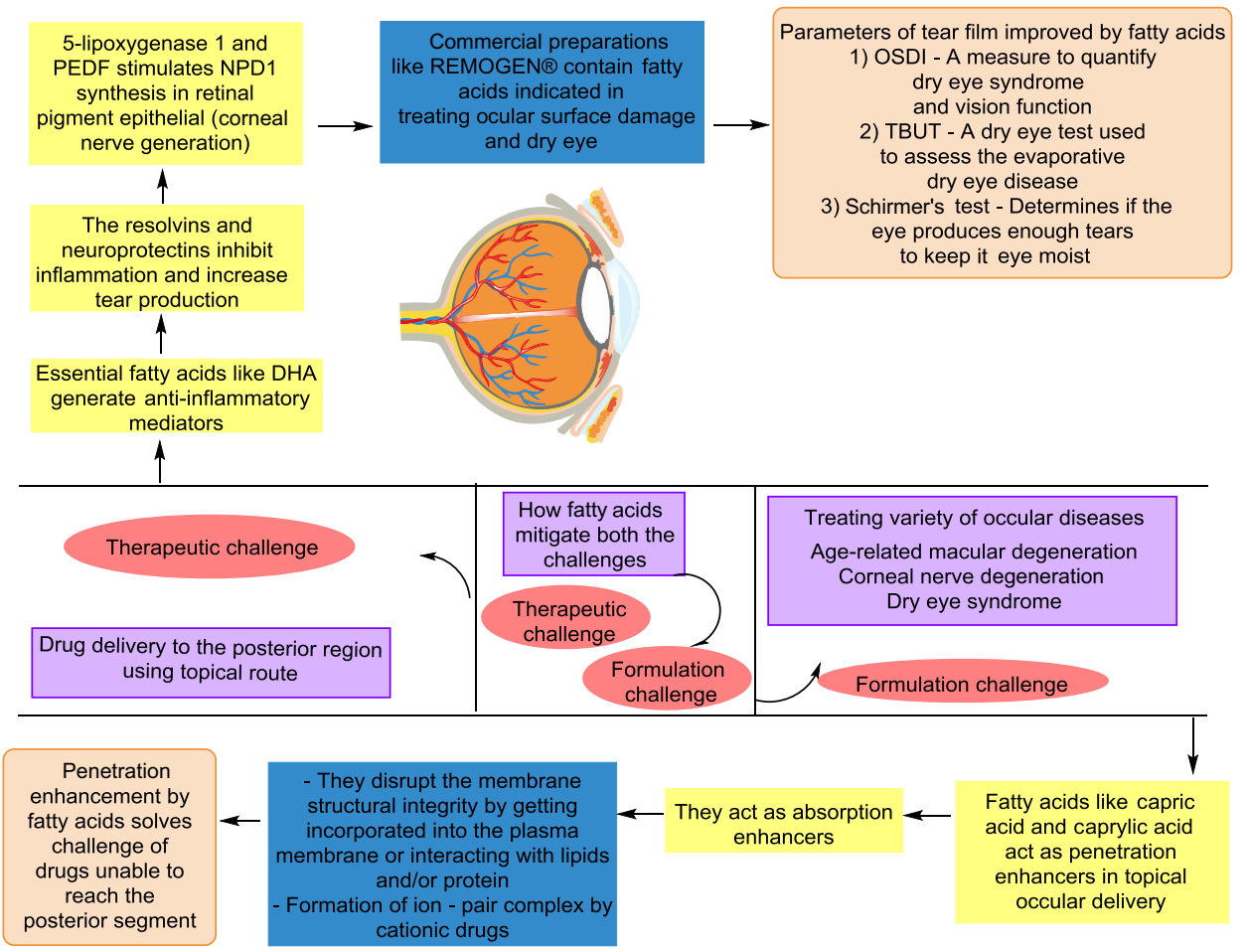


Fig. 8. Versatility of the FA, in mitigating the formulation as well as the therapeutic challenges posed by the anatomy of the eye for delivering the drugs to the posterior segment of eye. The upper portion describes how FAs successfully treat diseases from therapy viewpoint while the lower portion shows how FAs facilitated successful delivery of the formulation to posterior eye segments [41–43]. DHA, docosahexaenoic acid; FA, fatty acid; OSDI, Ocular Surface Disease Index; NPD1, neuroprotectin D1; PEDF, pigment epithelium-derived factor; TBUT, tear breakup time.

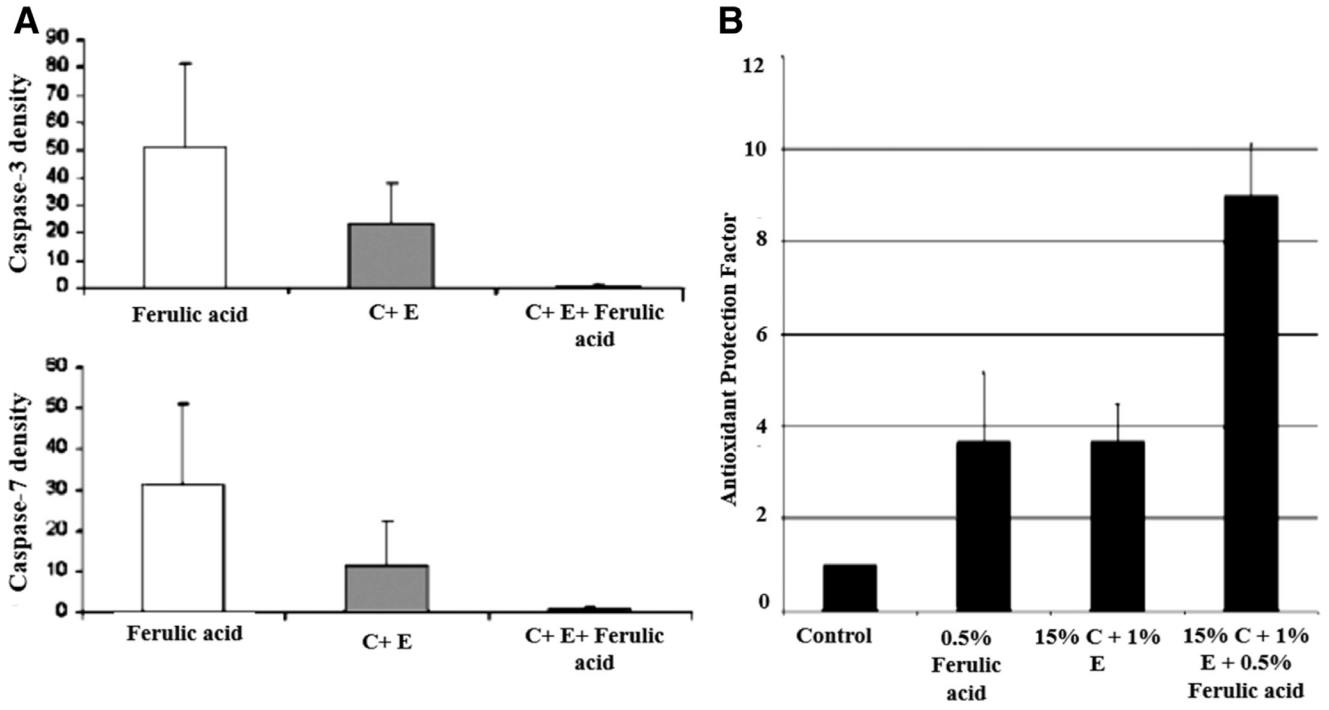


Fig. 9. (**A**) The graph shows the reduction in apoptosis markers after treatment with ferulic acid, vitamin C + vitamin E alone, and vitamin C + vitamin E + ferulic acid respectively. The highest reduction is seen on treatment with combination of vitamin C + vitamin E + ferulic acid. (**B**) The antioxidant protection factor was found to be highest with formulations comprising of 0.5% ferulic acid + 15% vitamin C + 1%. (Adapted from [45].)

Functionality of excipients play an important role in demonstrating action [44]. They have shown how a reduction in the number of formulation ingredients can be achieved by use of FAs in the formulation. A study conducted by Lin et al. [45] establishes the above-mentioned claims regarding FAs. Vitamins C and E are added because they help in protecting the fluids present in the body from sunlight. The cell membrane is also protected by such vitamins from the oxidative stress generated by harmful ultraviolet radiation. Vitamin C and E formulations are highly susceptible to degradation and do not reach a therapeutic amount at the site of action. FA-based agents such as tocopherol and ascorbic acid, when added to a formulation, help in improvement of the overall stability and also enhance the photoprotective effect of vitamins C and E. When 0.5% of ferulic acid, an agent derived from the cell wall present in plants, is added, an eight times increase in photoprotective activity of the formulation was seen compared with the earlier formulation. Ferulic acid improved the stability of the earlier formulation comprising of vitamins C and E and α-tocopherol. The authors also performed various studies such as lowering of the caspase-3 and caspase -7 levels, which are markers for reduction in apoptosis of keratinocytes with concomitant increase in antioxidant protection factor [45]. Figure 9 depicts the comparative evaluation of the antioxidant and photoprotective effect provided by different formulations comprising different ingredients.

Undecylic acid, which is a MUFA, is reported to exhibit virucidal activity. The researchers evaluated the antiviral properties of undecylic acid by conducting a large multicenter clinical trial comprising 573 patients. Of these patients, 293 were treated with undecylic acid and the remaining patients received placebos. The patients chosen for the study experienced orolabial herpes virus-mediated lip margin vesiculation at least three times a year. The studies revealed that the cream containing 15% undecylic acid was effective in reducing the viral shedding and was able to cure the disease when the formulation was applied at an early stage (i.e., the prodermal stage of the disease) [46].

There are numerous patents available in literature describing the dual purpose of FAs as an agent in formulation drug delivery and as a therapeutic aid [47]. Through their work, the authors showed that C_6–C_{18} FAs like lauric acid can act as a microbicidal lipid. The formulation in the form of hydrogel was highly effective in treatment of herpes simplex virus infections. The viruses, namely herpes simplex virus-1 and vesicular stomatitis virus (VSV), were inactivated by long-chain unsaturated and medium-chain saturated FA, whereas long-chain saturated and short-chain FA had no or very naive effect in killing these viruses. The derivative of FAs like 1-monoglyceride showed promising virucidal effect as compared with free FA (FFAs). The exact mechanism of virucidal effect of the FAs required further research; however, preliminary investigation using electron microscope showed leakage of the viral envelope of VSV and at higher concentrations a complete disintegration of the viral envelope. The FA-derivative capric acid 1-monoglyceride and lauric acid 1-monoglyceride were 10-fold more active than capric acid and lauric acids (i.e., the FFAs). Plasma proteins in the blood affect the activity of FAs, as the medium- and long-chain FAs were equally effective in virucidal effect in vitro but in blood due to stronger binding to plasma protein and less solubility long-chain FAs proved to be less effective than medium-chain FAs, which showed less binding and higher solubility. The specific FAs that are known to be effective virucidal agents are caproic acid (6:0), pelargonic acid (9:0) enanthic acid (7:0), and caprylic acid (8:0).

FAs can be used topically in a variety of ways. One such application of FA is in treatment of skin papilloma manipulated by the human papillomavirus (HPV). The structural specificity of OA has made it an automatic choice for stabilizing the natural protein α-lactalbumin, which is found in human milk . The study reveals a unique and safe way of treating skin tumors and provides a long-lasting cure for skin tumors. The discovery is serendipitous but involves keen observation. The lung cancer cell line showed decline in growth of tumor after accidental exposure to milk. The

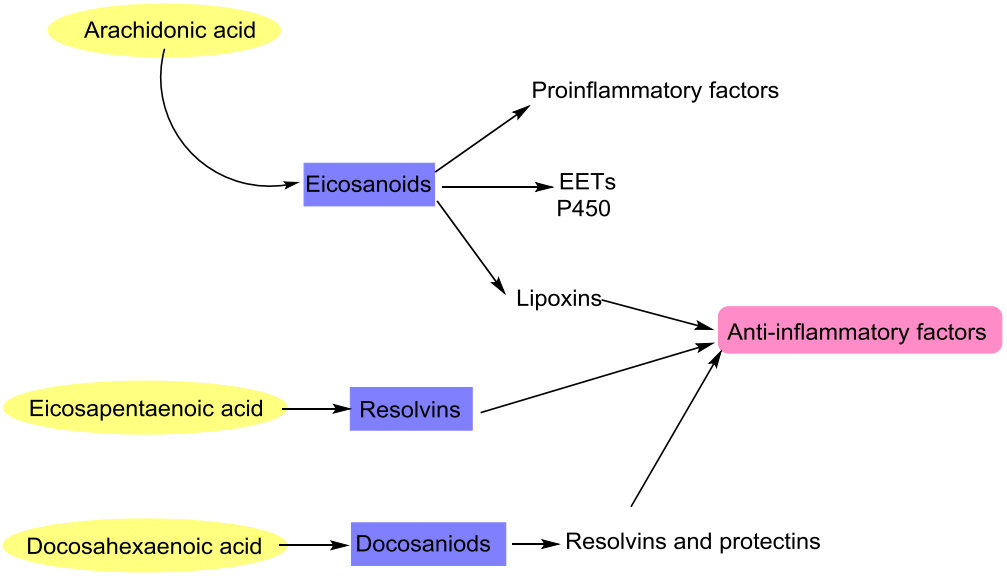


Fig. 10. Production pathways of mediators derived from long-chain polyunsaturated fatty acids. EET, epoxyeicosatrienoic acid.

component was identified as α-lactalbumin having a conformation stabilized by a lipid cofactor OA after extensive trials. The native structure of α-lactalbumin was modified by deliberate folding to finally generate the desired form, which was stabilized by OA (a lipid cofactor) [48].

The PUFAs have their own anti-inflammatory effects and concurrent administration of glucocorticoids can help decrease the dependence on glucocorticoids in dermatitis. Many studies have been reported regarding the effect of PUFAs in atopic dermatitis [49]. The use of PUFAs is legitimate due to the fact that in dogs affected with atopic dermatitis there is decreased conversion of ω-6 LAs to prostaglandins and also a Δ-6-desaturase deficiency. Various studies have been performed to determine the effect of PUFAs on atopic dermatitis and other diseases and the effect is substantial; however, the sole use of PUFAs may not guarantee complete cure [50].

FAs in treatment of inflammatory diseases

The human immune system represents a defense mechanism against infectious organisms and other environmental disturbances. The immune response comprises several steps that prevent entry of infectious organisms, identification of infectious organisms if they do invade, eliminate the invading infectious organisms, and retain the memory of the encounters. Use of FAs in humans has largely focused on the effects of long-chain PUFAs (LCPUFAs) on inflammation [51], mainly because lipid-derived mediators involved in the inflammatory response are produced from LCPUFA (mainly the ω-6 PUFA ARA and the ω-3 PUFA EPA and DHA). Mediators produced from these FAs are involved in both the activation and the resolution of the inflammatory process, as shown in Figure 10. Among the lipid mediators in inflammation, ARA is quantitatively the most important FA precursor of lipid mediators. Once released from the PL precursor, ARA is converted into different members of the eicosanoid family (prostaglandins, thromboxanes, leukotrienes, lipoxins, hydroxyl, and hydroperoxyeicosatetraenoic acids) by the sequential action of various enzymes, main among which are the cyclooxygenases and lipoxygenases. These enzymes are induced by different inflammatory stimuli and show different cellular distributions. More recently, analogous mediators derived from EPA (eicosanoids, resolvins, docosanoids) and DHA (resolvins, protectins) have been identified. Increased consumption of EPA and DHA in the diet can decrease the levels of ARA in cell membrane and phospholipid can also inhibit ARA metabolism. Tremendous research in this area has shown that eicosanoids produced from ARA are involved in many inflammatory conditions and ω-3 LCPUFAs decrease the production of eicosanoids from ARA, resulting in most clinical studies with a focus on the use of ω-3 LCPUFAs, usually in the form of fish oil, as a potential therapeutic agent. Studies using ω-3 LCPUFA supplementation have been conducted for a number of inflammatory diseases, but the evidence of beneficial effects appears to be greater for some of them (e.g., pediatric asthma, inflammatory bowel disease such as Crohn's disease and ulcerative colitis, and rheumatoid arthritis) [51, 52].

It has been reported that Achilles tendon healing can be promoted by the topical application of fish oil. According to a study performed by Chan et al. [53], topical application of fish oil in combination with ultrasound promoted collagen synthesis and hence connective tissue healing. On the basis of the study, it was observed that omega FAs proved helpful in increasing the structural stiffness of the repairing tendon with no effect on its strength. The omega FAs help in tendon healing mainly through two processes: The growth factors, like vascular endothelial growth factor (VEGF), are upregulated due to omega FA, which helps in tendon healing. Also the omega FAs stop the loss of glycosaminoglycans, which are very crucial for the process of tendon healing [53]. The versatility of FAs can again be seen from their broad spectrum of activity. The ω-3 PUFAs have been reported to promote collagen synthesis in tissues of porcine ligament fibroblasts, mouse fibroblasts, and rat cutaneous injuries.

FAs given topically have helped patients with osteoarthritis. A study in this regard was done by Kraemer et al. [54], who sought to determine the positive alteration in functional mobility of patients with osteoarthritis. The acetylated base comprises a blend of different FAs, consisting of cetylated FAs like cetyl myristate, cetyl laureate, cetyl palmitate, and cetyl oleate. The patients were analyzed on different parameters; namely, how well they were able to rotate their knee (range of motion), their ability to balance their body using their legs, and their ability to climb or descend a staircase. The patients showed stark improvement in their ability to climb stairs 30 min after the treatment, thus showing the ability of FAs to immediately ease the stiffness generated by osteoarthritis. The patient's ability to balance strength and improve endurance was evaluated by

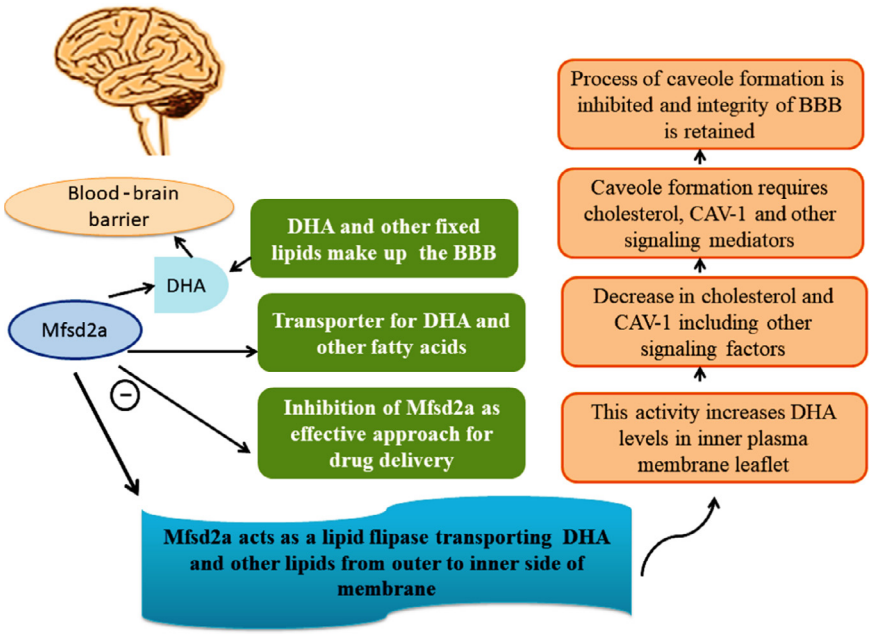


Fig. 11. The BBB composition is regulated by the DHA and inhibiting the DHA transporter Msfd2a can serve as an alternative way of enhancing the drug delivery across the BBB. BBB, blood–brain barrier; CAV, caveolin; DHA, docosahexaenoic acid.

performing the unilateral anterior reach test. Patients were able to easily flex and maintain balance after a treatment of 30 d. There was significant difference between the treated and placebo groups [54]. The ω-3 and ω-6 PUFAs can act in a contrasting manner. Recent animal studies confirmed a prominent reduction in inflammatory markers. Oxidative stress was seen in animals who were treated with ω-3 PUFAs, whereas ω-6 PUFAs reduced the expression of all these markers. Human intervention studies with ω-3 PUFAs have shown significant improvement in osteoarthritis and pain management but more robust studies with well-defined endpoints are needed to confirm the benefits of ω-3 PUFAs [55].

Role of FAs in treatment of ailments affecting the CNS

With the increase in the aging population, there is a rise in the prevalence of dementia worldwide. The most common cause is Alzheimer's disease (AD), considered an irreversible condition at present [56]. After ingestion, PUFAs are distributed to cells and get enriched in cellular membranes, where they are responsible for cellular metabolism and survival. They are known to contribute to the physical-chemical properties of the cell membrane by affecting membrane permeability and, hence the transport functions and associated ion channels. ω-3 DHA is essential for proper functioning and development of the brain and is the main ω-3 PUFA present in the brain [57].

Role of FAs in circumventing the blood–brain barrier and mechanism involved

Disorders of the brain have been creating havoc for mankind for centuries and new diseases continually plague researchers around the globe. FAs have provided an alternative and relatively safer approach to treat these ailments. FAs earlier in this review were reported as dual agents that can provide a therapeutic edge and also act as an excipient to improve the delivery of the drugs across the brain. Transport of FAs has long been debated and the general consensus is that the FAs are transported by both the mechanisms, that is, the passive diffusion (flip-flop model, which keeps at bay the involvement of specific transporters for FAs) and the use of FA transporters, namely FA transporter protein (FATP 1-6), intracellular FA binding proteins (FABP 1-9), caveolin, and plasma membrane FA binding protein (FABP pm). The mechanism for FA transport across the blood–brain barrier (BBB) received a huge boost with the discovery of superfamily of Mfsd2a (orphan transporters). Mfsd2a acts as a chief transporter, which transfers DHA across the BBB [58,59]. This striking discovery, which was published by Nguyen et al. [60], indicates that Mfsd2a knockout mice show astounding reduction in levels of lysophosphatidylcholine (LPC) DHA but not in the form of unesterified FA. FA that are not more than 14–carbonyl acyl chain, not just DHA but also LPC oleate and LPC palmitate, are transported. The study shows that the knockout mice have markedly decreased concentrations of the labeled protocadherin alpha gene and another LPC. The FA transporter Mfsd2a blockade results in the entry of therapeutic agent inside the brain [60]. The process of transcytosis (i.e., caveole formation: a type of vesicle that is formed when the segment of the cell membrane pinches itself off) is stopped by the Mfsd2a transporter. DHA has been proven to comprise a large part of the membrane that makes up the brain and BBB. The role of DHA in maintaining and altering the rigidity of the BBB is proven repeatedly. Figure 11 describes how DHA alters the BBB and, indirectly, the transport of drugs across the BBB.

DHA is essential for the integrity of the BBB, but are in contrast to the fact FAs have been traditionally reported to act as penetration enhancer in the formulations. Numerous reports show them as having varied mechanisms for effective penetration enhancement [61]. A recent study published by Khunt et al. [62] mentions the role of butter oil as a novel penetration enhancer for central nervous system (CNS) delivery of the drug quetiapine fumarate via the intranasal route. The study highlights the fact that butter oil is a blend of numerous FAs and FA traditionally have been shown to promote penetration of numerous drugs by disruption of the cell membrane or by interacting with lipids present in the cell membrane. The FAs present in butter oil are reported to be OA, LA, capric acid, lauric acid, myristic acid, and butyric acid. These FAs per se, have been reported to be penetration enhancers and are

purported to improve the penetration of various drugs across the BBB. Butter oil contains a blend of these FAs and has been hypothesized to further improve the penetration of drugs higher than individual FAs. The authors also reported that butter oil is able to inhibit the P-glycoprotein efflux pump, which is present in the BBB and which acts as a major component to efflux the drugs from the brain. The histopathologic studies and interaction studies proved that borage oil is a safe and non-toxic penetration enhancer. The bioavailability of the drug under consideration was found to be 4.6 times higher inside the brain when compared with the drug solution alone via the intranasal route, which proves that butter oil comprising the numerous FAs helped increase the permeation of the drug inside the brain [62].

CNS disorder: How FAs are changing the way these disorders are treated

The omega FAs have been touted to be useful in many CNS disorders owing to their role in cell signaling. The ω-3 FAs, however, have no significant clinical trials backing their claim, and the methodology used to carry out these studies is not appropriate. The ω-3 FAs comprising mainly DHA and EPA can be used in the capacity of adjunctive therapy and not as a therapy on which the patient can solely rely. Various disorders like attention deficit/hyperactivity disorder (ADHD), Alzheimer's disease, multiple sclerosis, and schizophrenia, in addition to eating disorders like anorexia nervosa, have been studied for the effect of omega FAs [63,64]. The omega FAs in literature have been proven effective in the treatment of bipolar disorders, mild forms of ADHD, and to some extent may help to increase the cognitive function of healthy aging adults [65,66].

There are numerous studies with many controversial results regarding the effects on ADHD. In a randomized, double-blind, placebo-controlled study performed by Stoll et al. [67] was conducted to show the potential of FAs in patients with ADHD. The patients chosen for the study had mild to moderate ADHD-1, according to selection criteria in the fourth edition of the Diagnostic and Statistical Manual (DSM-4). The DSM-4 is a set of requirements for a person to be diagnosed with ADHD and specifies that the person must have symptoms of ADHD persisting for ≥6 mo to be characterized as a development that is not consistent with a normal individual. The study was to be conducted in two phases: Phase 1 was the double-blind evaluation of omega versus placebo administration and phase 2 was the open-label treatment in which all the volunteers were given ω-3/6 supplements. Only preliminary analysis suggests that the ω-3/6 supplements had a definite effect on the ADHD-RS total score (a reliable assessment tool used to diagnose ADHD, which uses a set of questionnaires for parents and teachers containing 18 questions regarding the child's hyperactivity). The supplement failed to show any benefit on the ADHD-RS hyperactivity scale and the Child Depression Rating Scale, which is conducted with children 6 to 12 y of age to check the severity of depression. Results of these assessments are critical to prove any benefit of ω-3/6 supplementation. The meta-analyses done for all the reported clinical trials revealed a need to fine-tune the methodologies involved in the randomized clinical trials performed using ω-3/6 supplements. They also imply that omega FAs can only be used as adjunctive therapy for ADHD with little clinical significant effects on reducing the emotional liability, conduct, and inattentiveness of children. They further state the need to conduct studies with a large sample size and with higher doses of EPA because DHA proved to be less effective in the case of ADHD symptoms than DHA. Patients with bipolar disorders can get some respite from supplementation with FA as an adjunctive therapy.

One of the studies set out to determine whether FAs possess the same mood-stabilizing properties as lithium carbonate and valproate because FAs have similar signal transduction pathways as these drugs, which are poster drugs for bipolar disorders. The study was conducted for 4 mo and included the established treatment, placebo (olive oil), and FA supplementation in 30 patients. The DSM-4 was used as a diagnostic tool for the mood disorders and the volunteers were at least required to have one manic or hypomanic episode in the previous year before being enrolled in the study. The outcome was evaluated based on the standard rating scales like the Young Mania Rating Scale (YMRS), Hamilton Rating Scale, Clinical Global Rating Scale, and Global Assessment Scale and a brief Adverse Effect Rating Scale. The results reveal considerable difference between the FA-supplemented group and the placebo (olive oil) group with reference to each rating scale. The conclusion obtained revealed staggering benefits via the treatment of ω-3 FAs. The mechanism of action of FAs was found to be similar to valproate, lithium bicarbonate via the suppression of postsynaptic signal transduction. Although on a small scale, this study justifies the use of omega FAs as an adjunctive therapy to curb the overall dependence on lithium carbonate and valproate [67].

The effect of ω-3 FAs on psychosis was investigated owing to the body of evidence and the various neuro-signaling pathways with which it is involved. The individuals, who fall under the ultra-high-risk category, are susceptible psychosis; however, if aptly treated, they will not experience psychosis. One such treatment involves supplementation with ω-3 FAs. A study in this regard was published by Amminger et al. [68]. In a 2010 randomized placebo-controlled trial based on a 12-wk supplementation of ω-3 FAs, the same author evaluated the long-term effects of the 12-wk brief supplementation. The study was done for the duration of 6.7 y with volunteers who had been treated for 12 wk with ω-3 FAs. The results from both these studies seem quite promising. The main objective was to see the transition to psychosis and to study the effect on the positive and negative symptoms of psychosis. The primary outcome measure was transition to psychotic disorder. Secondary outcomes included symptomatic and functional changes. Of 81 participants, 76 (93.8%) completed the intervention. The study, which was carried out for 12 mo only had 2 drop outs (4.9%) from the treated group. The difference between the placebo-treated group and the ω-3 FA-treated group was 22.6% (95% confidence interval). There was also significant reduction in the positive ($P = 0.01$) and negative symptoms ($P = 0.02$) for the treated group compared with the placebo group. These findings at a preliminary stage conclude that ω-3 FA supplementation helped to stop the progression to psychosis. The effect of this 12-wk intervention caused short-term relief and a long-term effect was investigated by the same authors to obtain conclusive evidence regarding ω-3 FA therapy. They concluded that risk for progression to psychotic disorders and psychiatric morbidity declined and functional impairment was not seen during the follow-up, with no severe functional impairment [68,69]. The reproducibility of these clinical trials was conducted by McGorry et al. [70] in a study called NEURAPRO for a period of 6 mo with the same set of primary and secondary outcomes as that of Amminger et al. [68]. They also took into consideration the effect of FAs in combination with the established treatments of cognitive behavioral case management (CBCM) versus the effect of placebo plus CBCM treatment. The study was also carried out to check the general stages of functioning and psychopathology in individuals based on various rating scales like Brief Psychiatric Rating Scale (BPRS; range, 24–168), Scale for the Assessment of Negative Symptoms (SANS; range, 0–125), Montgomery-Åsberg Depression Rating Scale (MADRS; range, 0–60), YMRS (range, 0–44), Social and Occupational

Functioning Assessment Scale (SOFA; range, 0–100), and the Global Functioning: Social and Role scale (range, 0–10). For SOFA and Global Functioning: Social and Role scale, higher scores were better; for other measures, lower scores were better [70]. The extra virgin olive oil (EVOO), which is the purest form of olive oil and contains a rich mix of FAs namely OA ≤83%, palmitic acid, and LA) has been indicated in various learning and other memory-related disorders. Farr et al. [71] studied the effects of olive oil on learning in SAMP8 mice, which shows progressive rise in the Aβ plaque as the animal grows old. They also present age-associated behavioral impairments including learning and memory difficulties. The study was performed using coconut oil, butter, and EVOO. Coconut oil and butter are used because they also are reported to contain FAs and help improve learning and memory. The SAMP8 mice were administered butter, coconut oil, and EVOO for 6 wk. The mice were subjected to various T-maze tests, foot shock avoidance tests, and novel object identification tests. The comparative tests using all the above three agents revealed that EVOO-administered mice showed better retention times in the T-maze test than the mice administered the butter. EVOO-administered mice also showed an increase in glutathione reductase activity, hinting at reduced oxidative stress as a probable pathway [71]. PUFAs can be used as adjunctive therapy in combination with established therapies to reduce the dosage of the primary therapy.

The journey of FA-based products from clinical trials to bedside: Not all hunky-dory

The preceding section mentioned that FAs can be useful as a formulation adjuvant in drug delivery and also for treating various diseases. Numerous products containing FAs, such as capsules containing omega fish oil (rich in DHA and EPA), are available on the market and are prescribed by doctors for treatment of various ocular, CNS, and CVDs. Although use of FAs as formulation aid in drug delivery has been widely reported, there remain many grey areas that need further debate and thorough investigation.

Fas, namely ω-3 PUFAs, may exert harmful effects like blood thinning, immunosuppression in healthy patients, and conversion into potential procarcinogens [72]. PUFAs act as immunosuppressants, which can be helpful in patients with hypersensitivity but at the same time may cause adverse effects in healthy humans. It is reported that PUFAs do so mainly by inhibiting T-cell proliferation, thereby modulating various signaling proteins, which get activated on engagement of the T-cell receptor (TCR) and costimulatory molecules. This sort of PUFA modulation of T cells is reported to disrupt the formation of the immunologic synapse [73]. It is further reported to lower the surface expression of HLA class II molecules with simultaneous reduction in antigen presentation [73]. Diets rich in MUFAs are popularly believed to prevent the development of diabetes, but Plötz et al. [73] claimed that MUFA oleate and other FAs having C chain >14 carbons generate hydrogen peroxide, which is toxic to the pancreatic human β cells. MUFA oleate may act as a major physiologic toxin for pancreatic β cells [74]. It also has been reported that consumption of fish oil causes pathogenic infection [73].

Clinicians around the globe recommend the use of omega-enriched products to provide symptomatic relief for dry eyes and various ocular conditions. However, a recent study published in *The New England Journal of Medicine* has raised doubts over beneficial effects of fish-derived ω-3 EPA and DHA for ocular conditions. The DREAM (Dry Eye Assessment and Management) trial, involving ~349 patients, had mean change in OSDI score from the baseline as the primary outcome with Schrimer's test and conjunctival staining score as secondary outcome. The patients were treated with 3000 mg oral dose of fish-derived ω-3 EPA and DHA as treatment or olive oil (as placebo control). The DREAM study found that ω-3 supplements provide no significant difference in OSDI score and other secondary outcomes when compared with placebo-treated patients [75]. This study created a huge question mark concerning the prevalent use of such FA-based products for ocular conditions.

The American Heart Association recommends consumption of fish for prevention of CVDs. Such recommendations by various guidelines around the world came under review when systematic meta-analyses of all significant and non-significant clinical trials studying the effect of FA-based supplements on prevention of CVD and related mortality. The meta-analysis concluded that increasing EPA and DHA had minimal effect on mortality and heart-related ailments, although the use of EPA and DHA did raise the level of serum triacylglycerides slightly and increased high-density lipoprotein levels. These changes were insignificant and were of not much help to patients with CVD. Consumption of ω-3 capsules does not reduce heart disease, stroke, or death. Supplementary ω-3 fats are probably not useful for preventing or treating heart and circulatory diseases. The authors hinted that increasing use of plant-based ALA may be protective for some heart and circulatory diseases, but shatters the common belief that FAs aid in preventing heart ailments [76].

Despite increasing evidence from observational studies of a link between dietary fat intake and risk for dementia or cognitive decline, published randomized controlled trials (RCTs) do not offer conclusive evidence about beneficial effects. In one such reported single-blind RCT, the efficacy of ω-3 PUFAs in demented patients [77,78] showed a positive effect of joint PUFA (with a ω-6/ω-3 ratio = 4.5) and vitamin E supplementation on memory, mood, and appetite of 100 patients with Alzheimer's disease as reported by their caregivers [77]. However, this small trial had several methodological flaws. Another RCT was conducted in elderly Japanese suffering from dementia with thrombotic cerebrovascular disease, wherein the intervention group showed significant improvements on the dementia rating scale and Mini Mental Status Examination, but again the study was conducted with a very small sample, making it difficult to extrapolate to large numbers [78].

These studies and problems associated with use of FA-based products highlight the need for more rigorous and uniform (dose of FA and clinical outcomes) clinical trials. This, however, does not rule out that FA-based products do not have any substance or promise. It shows that scientists should ponder more over the strategies used to conduct clinical trials for FA-based products. Finally, there are many aspects of the interactions between PUFAs and body tissues that are worthy of further investigations and demands a lot of groundwork till we get definitive answers.

Conclusion

This review has shown the multifaceted nature of FA through various studies and emphasized the potential effect of their structure on various activities like membrane fluidity, formulation stability, and so on. The role of FAs in formulation is currently limited to their penetration-enhancing effect for delivering drugs to the posterior regions of the eye and to circumvent the BBB. FAs, although extensively investigated as therapeutic additives, are still used for their additive effect and cannot be used as a sole therapeutic agent. FAs to date have not been established as lone therapeutic agents, but more extensive studies are needed as they can offer potential solution for many ailments. The concentration of the FA required to show a therapeutic benefits also needs to be optimized and a uniform value has to be reported. The different and numerous ratios of ω-3 to ω-6 FAs need to be researched further to obtain a uniform value. The FA therapeutic benefits in CNS disorders like ADHD, Alzheimer's disease, and psychosis need further research

before they can be established as significant contributors in treating such diseases. The FAs also need to be researched as agents that can significantly alter oxidative stress, either positively or negatively. Their stability needs to be addressed if they have to be proven as agents that will contribute in reducing the oxidative stress. This review suggests that there is a dire need to study how different blends of FA can effect etiology of different diseases and potentially affect their treatment strategies.

References

[1] Nettleton JA. Omega-3 fatty acids and health. Boston, MA: Springer Science & Business Media; 1995.
[2] Spector AA, Kim H-Y. Discovery of essential fatty acids. J Lipid Res 2015;56:11–21.
[3] Lei E, Vacy K, Boon WC. Fatty acids and their therapeutic potential in neurological disorders. Neurochem Int 2016;95:75–84.
[4] Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti–inflammatory potential of long–chain omega–3 fatty acids. Nutr Rev 2010;68:280–9.
[5] Yang X, Sheng W, Sun GY, Lee JCM. Effects of fatty acid unsaturation numbers on membrane fluidity and α-secretase-dependent amyloid precursor protein processing. Neurochem Int 2011;58:321–9.
[6] Castuma CE, Brenner RR. The influence of fatty acid unsaturation and physical properties of microsomal membrane phospholipids on UDP-glucuronyltransferase activity. Biochem J 1989;258:723–31.
[7] Yazdi S, Stein M, Elinder F, Andersson M, Lindahl E. The molecular basis of polyunsaturated fatty acid interactions with the shaker voltage-gated potassium channel. PLoS Comput Biol 2016;12:e1004704.
[8] Lenin R, Joy PA. Studies on the role of unsaturation in the fatty acid surfactant molecule on the thermal conductivity of magnetite nanofluids. J Colloid Interface Sci 2017;506:162–8.
[9] Philip J, Shima P, Raj B. Enhancement of thermal conductivity in magnetite based nanofluid due to chainlike structures. Appl Phys Lett 2007;91:203108.
[10] Altan CL, Elkatmis A, Yüksel M, Aslan N, Bucak S. Enhancement of thermal conductivity upon application of magnetic field to Fe3O4 nanofluids. J Appl Phys 2011;110:093917.
[11] Cordero-Morales JF, Vásquez V. How lipids contribute to ion channel function, a fat perspective on direct and indirect interactions. Curr Opin Struc Biol 2018;51:92–8.
[12] Elinder F, Liin SI. Actions and mechanisms of polyunsaturated fatty acids on voltage-gated ion channels. Front Physiol 2017;8:43.
[13] Eldho NV, Feller SE, Tristram-Nagle S, Polozov IV, Gawrisch K. Polyunsaturated docosahexaenoic vs docosapentaenoic acids: differences in lipid matrix properties from the loss of one double bond. Brain Res 1995;669:225–33.
[14] Feller SE, Gawrisch K, MacKerell AD. Polyunsaturated fatty acids in lipid bilayers: intrinsic and environmental contributions to their unique physical properties. J Am Chem Soc 2002;124:318–26.
[15] Shahidi F, Ambigaipalan P. Omega-3 polyunsaturated fatty acids and their health benefits. Ann Rev Food Sci Technol 2018;9.
[16] Tian Y, Aursnes M, Hansen TV, Tungen JE, Galpin JD, Leisle L, et al. Atomic determinants of BK channel activation by polyunsaturated fatty acids. Proc Natl Acad Sci U S A 2016;113:13905–10.
[17] Ghate D, Edelhauser HF. Ocular drug delivery. Expert Opin Drug Deliv 2006;3:275–87.
[18] Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. AAPS J 2010;12:348–60.
[19] Shah SS, Denham LV, Elison JR, Bhattacharjee PS, Clement C, Huq T, et al. Drug delivery to the posterior segment of the eye for pharmacologic therapy. Expert Rev Ophthalmol 2010;5:75–93.
[20] Awwad S, Ahmed M, Abeer H, Sharma G, Heng JS, Khaw PT, et al. Principles of pharmacology in the eye. Br J Pharmacol 2017;174:4205–23.
[21] Lieberman S, Enig MG, Preuss HG. A review of monolaurin and lauric acid: natural virucidal and bactericidal agents. Altern Complement Ther 2006;12:310–4.
[22] Chanda W, Joseph TP, Guo X-f, Wang W-d, Liu M, Vuai MS, et al. Effectiveness of omega-3 polyunsaturated fatty acids against microbial pathogens. J Zhejiang Univ Sci B 2018;19:253–62.
[23] Yoon BK, Jackman JA, Valle-González ER, Cho N-J. Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. Int J Mol Sci 2018;19:1114.
[24] Saccà SC, Cutolo CA, Ferrari D, Corazza P, Traverso CE. The eye, oxidative damage and polyunsaturated fatty acids. Nutrients 2018;10:668.
[25] Chong EW, Kreis AJ, Wong TY, Simpson JA, Guymer RH. Dietary ω-3 fatty acid and fish intake in the primary prevention of age-related macular degeneration: a systematic review and meta-analysis. Arch Ophthalmol 2008;126:826–33.
[26] Tamilvanan S, Benita S. The potential of lipid emulsion for ocular delivery of lipophilic drugs. Eur J Pharm Biopharm 2004;58:357–68.
[27] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318–27.
[28] Churchward CP, Alany RG, Kirk RS, Walker AJ, Snyder LA. Prevention of ophthalmia neonatorum caused by *Neisseria gonorrhoeae* using a fatty acid-based formulation. mBio 2017;8. e00534–17.
[29] Butt U, ElShaer A, Snyder LA, Al-Kinani AA, Le Gresley A, Alany RG. Fatty acid based microemulsions to combat ophthalmia neonatorum caused by *Neisseria gonorrhoeae* and *Staphylococcus aureus*. Nanomaterials 2018;8:51.
[30] Jager RD, Aiello LP, Patel SC, Cunningham Jr ET. Risks of intravitreous injection: a comprehensive review. Retina 2004;24:676–98.
[31] Wadhwa S, Paliwal R, Paliwal SR, Vyas S. Nanocarriers in ocular drug delivery: an update review. Curr Pharm Des 2009;15:2724–50.
[32] Raval N, Khunt D, Misra M. Microemulsion-based delivery of triamcinolone acetonide to posterior segment of eye using chitosan and butter oil as permeation enhancer: an in vitro and in vivo investigation. J Microencapsul 2018;35:62–77.
[33] Zambito Y, Di Colo G. Chitosan and its derivatives as intraocular penetration enhancers. J Drug Del Sci Technol 2010;20:45–52.
[34] Calder PC, Yaqoob P, Harvey DJ, Watts A, Newsholme EA. Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity. Biochem J 1994;300:509–18.
[35] SanGiovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Prog Retin Eye Res 2005;24:87–138.
[36] Roncone M, Bartlett H, Eperjesi F. Essential fatty acids for dry eye: a review. Cont Lens Anterior Eye 2010;33:49–54.
[37] Barabino S, Horwath-Winter J, Messmer EM, Rolando M, Aragona P, Kinoshita S. The role of systemic and topical fatty acids for dry eye treatment. Prog Retin Eye Res 2017;61:23–34.
[38] He J, Bazan HE. Omega-3 fatty acids in dry eye and corneal nerve regeneration after refractive surgery. Prostaglandins Leukot Essent Fatty Acids 2010;82:319–25.
[39] Mohammadpour M, Mehrabi S, Hassanpoor N, Mirshahi R. Effects of adjuvant omega-3 fatty acid supplementation on dry eye syndrome following cataract surgery: a randomized clinical trial. J Curr Ophthalmol 2017;29:33–8.
[40] Savla R, Browne J, Plassat V, Wasan KM, Wasan EK. Review and analysis of FDA approved drugs using lipid-based formulations. Drug Dev Ind Pharm 2017;43:1743–58.
[41] Rashid S, Jin Y, Ecoiffier T, Barabino S, Schaumberg DA, Dana MR. Topical omega-3 and omega-6 fatty acids for treatment of dry eye. Arch Ophthalmol 2008;126:219–25.
[42] Pinazo-Durán MD, Galbis-Estrada C, Pons-Vázquez S, Cantú-Dibildox J, Marco-Ramírez C, Benítez-del-Castillo J. Effects of a nutraceutical formulation based on the combination of antioxidants and ω-3 essential fatty acids in the expression of inflammation and immune response mediators in tears from patients with dry eye disorders. Clin Interv Aging 2013;8:139.
[43] Calder PC. n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006;83:1505S–19S.
[44] Sharma K, Thakkar S, Khurana S, Bansal AK. Excipients and their functionality for enabling technologies in oral dosage forms. Wiley Online Library 2016. In Pharmaceutical Excipients, O. M. Koo (Ed.). https://doi.org/10.1002/9781118992432.ch3.
[45] Lin F-H, Lin J-Y, Gupta RD, Tournas JA, Burch JA, Selim MA, et al. Ferulic acid stabilizes a solution of vitamins C and E and doubles its photoprotection of skin. J Investig Dermatol 2005;125:826–32.
[46] Shafran SD, Sacks SL, Aoki FY, Tyrrell DL, Schlech III WF, Mendelson J, et al. Topical undecylenic acid for herpes simplex labialis: a multicenter, placebo-controlled trial. J Infect Dis 1997;176:78–83.
[47] Thormar H, Kristmundsdottir T. Topical formulations containing as a therapeutic active agent fatty acids or fatty alcohols or monoglyceride derivatives thereof for treating of mucosa infections. Google Patents 2003.
[48] Gustafsson L, Leijonhufvud I, Aronsson A, Mossberg A-K, Svanborg C. Treatment of skin papillomas with topical α-lactalbumin–oleic acid. N Eng J Med 2004;350:2663–72.
[49] Cerrato S, Ramió-Lluch L, Fondevila D, Rodes D, Brazis P. Effects of essential oils and polyunsaturated fatty acids on canine skin equivalents: skin lipid assessment and morphological evaluation. Journal of Veterinary Medicine 2013;2013.
[50] Blaskovic M, Rosenkrantz W, Neuber A, Sauter-Louis C, Mueller R. The effect of a spot-on formulation containing polyunsaturated fatty acids and essential oils on dogs with atopic dermatitis. Vet J 2014;199:39–43.
[51] Tretter S, Mueller RS. The influence of topical unsaturated fatty acids and essential oils on normal and atopic dogs. J Am Anim Hosp Assoc 2011;47:236–40.
[52] Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids 2006;75:197–202.
[53] Chan KO, Tong HH, Ng GY. Topical fish oil application coupling with therapeutic ultrasound improves tendon healing. Ultrasound Med Biol 2016;42:2983–9.
[54] Kraemer WJ, Ratamess NA, Anderson JM, Maresh CM, Tiberio DP, Joyce ME, et al. Effect of a cetylated fatty acid topical cream on functional mobility and quality of life of patients with osteoarthritis. J Rheumatol 2004;31:767–74.

[55] Loef M, Schoones J, Kloppenburg M, Ioan-Facsinay A. Fatty acids and osteoarthritis: different types, different effects [Epub ahead of print]. Joint Bone Spine 2018.
[56] Barberger-Gateau P. PUFAs and risk of cognitive decline or dementia: epidemiological data. Ol Corps Gras Lipides 2007;14:198–201.
[57] Saccà S, Cutolo C, Ferrari D, Corazza P, Traverso C. The eye, oxidative damage and polyunsaturated fatty acids. Nutrients 2018;10:668.
[58] Mitchell RW, Hatch GM. Fatty acid transport into the brain: of fatty acid fables and lipid tails. Prostaglandins Leukot Essent Fatty Acids 2011;85:293–302.
[59] Ochiai Y, Uchida Y, Ohtsuki S, Tachikawa M, Aizawa S, Terasaki T. The blood–brain barrier fatty acid transport protein 1 (FATP1/SLC27A1) supplies docosahexaenoic acid to the brain, and insulin facilitates transport. J Neurochem 2017;141:400–12.
[60] Nguyen LN, Ma D, Shui G, Wong P, Cazenave-Gassiot A, Zhang X, et al. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. Nature 2014;509:503–6.
[61] Andreone BJ, Chow BW, Tata A, Lacoste B, Ben-Zvi A, Bullock K, et al. Blood-brain barrier permeability is regulated by lipid transport-dependent suppression of caveolae-mediated transcytosis. Neuron 2017;94:581–94. e5.
[62] Khunt D, Shah B, Misra M. Role of butter oil in brain targeted delivery of Quetiapine fumarate microemulsion via intranasal route. J Drug Del Sci Tech 2017;40:11–20.
[63] Banaschewski T, Belsham B, Bloch MH, Ferrin M, Johnson M, Kustow J, et al. Supplementation with polyunsaturated fatty acids (PUFAs) in the management of attention deficit hyperactivity disorder (ADHD) [Epub ahead of print]. Nutrition Health 2018.
[64] Shinto L, Marracci G, Mohr DC, Bumgarner L, Murchison C, Senders A, et al. Omega-3 fatty acids for depression in multiple sclerosis: a randomized pilot study. PLoS One 2016;11:e0147195.
[65] Bo Y, Zhang X, Wang Y, You J, Cui H, Zhu Y, et al. The n-3 polyunsaturated fatty acids supplementation improved the cognitive function in the Chinese elderly with mild cognitive impairment: a double-blind randomized controlled trial. Nutrients 2017;9:54.
[66] Nadjar A, Leyrolle Q, Joffre C, Layé S. Antiinflammatory properties of dietary n-3 polyunsaturated fatty acids protect against cognitive decline in aging and neurodegenerative diseases. In: Farooqui T, Farooqui Aa, eds. Role of the Mediterranean diet in the brain and neurodegenerative diseases, London, UK: Academic Press; 2018.
[67] Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E, et al. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 1999;56:407–12.
[68] Amminger GP, Schäfer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, et al. Long-chain ω-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch Gen Psychiatry 2010;67:146–54.
[69] Amminger GP, Schäfer MR, Schlögelhofer M, Klier CM, McGorry PD. Longer-term outcome in the prevention of psychotic disorders by the Vienna omega-3 study. Nature Comm 2015;6.
[70] McGorry PD, Nelson B, Markulev C, Yuen HP, Schäfer MR, Mossaheb N, et al. Effect of ω-3 polyunsaturated fatty acids in young people at ultrahigh risk for psychotic disorders: the NEURAPRO randomized clinical trial. JAMA Psychiatry 2017;74:19–27.
[71] Farr SA, Price TO, Dominguez LJ, Motisi A, Saiano F, Niehoff ML, et al. Extra virgin olive oil improves learning and memory in SAMP8 mice. J Alzheimers Dis 2012;28:81–92.
[72] Serini S, Fasano E, Piccioni E, Cittadini AR, Calviello G. Dietary n-3 polyunsaturated fatty acids and the paradox of their health benefits and potential harmful effects. Chem Res Toxicol 2011;24:2093–105.
[73] Shaikh SR, Edidin M. Immunosuppressive effects of polyunsaturated fatty acids on antigen presentation by human leukocyte antigen class I molecules. J Lipid Res 2007;48:127–38.
[74] Plötz T, Krümmel B, Laporte A, Pingitore A, Persaud S, Jörns A, et al. The monounsaturated fatty acid oleate is the major physiological toxic free fatty acid for human beta cells. Nutr Diabetes 2017;7:305.
[75] The Dry Eye Assessment and Management Study Research Group. n-3 fatty acid supplementation for the treatment of dry eye disease. N Eng J Med 2018;378:1681–90.
[76] Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, et al. Omega−3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev 2018;7:CD003177.
[77] Yehuda S, Rabinovtz S, Carasso RL, Mostofsky DI. Essential fatty acids preparation (SR-3) improves Alzheimer's patients quality of life. Int J Neurosci 1996;87:141–9.
[78] Terano T, Fujishiro S, Ban T, Yamamoto K, Tanaka T, Noguchi Y, et al. Docosahexaenoic acid supplementation improves the moderately severe dementia from thrombotic cerebrovascular diseases. Lipids 1999;34:S345–S6.
[79] Chow CK. Fatty acids in foods and their health implications. Boca Raton, FL: CRC Press; 2007.
[80] Akoh CC. Food lipids: chemistry, nutrition, and biotechnology. Boca Raton, FL: CRC Press; 2017.
[81] Owen R. Paliperidone palmitate injection: Its efficacy, safety and tolerability in schizophrenia. Drug Today 2010;46:463–71.
[82] Zuin A, Cousseau T, Sinatora A, Toma SH, Araki K, Toma HE. Lipophilic magnetite nanoparticles coated with stearic acid: a potential agent for friction and wear reduction. Tribol Int 2017;112:10–9.
[83] Noor NM, Sheikh K, Somavarapu S, Taylor KM. Preparation and characterization of dutasteride-loaded nanostructured lipid carriers coated with stearic acid-chitosan oligomer for topical delivery. Eur J Pharm Biopharm 2017;117:372–84.
[84] Layek B, Singh J. Caproic acid grafted chitosan cationic nanocomplexes for enhanced gene delivery: effect of degree of substitution. Int J App Pharm 2013;447:182–91.
[85] Yamamoto A, Setoh K, Murakami M, Shironoshita M, Kobayashi T, Fujimoto K, et al. Enhanced transdermal delivery of phenylalanyl-glycine by chemical modification with various fatty acids. Int J Pharm 2003;250:119–28.
[86] Kumar UD, Christopher V, Sobarani D, Sastry NY. Lauric acid as potential natural product in the treatment of cardiovascular disease: a review. J Bioanal Biomed 2014;6:37.
[87] Nakatsuji T, Kao MC, Fang J-Y, Zouboulis CC, Zhang L, Gallo RL, et al. Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. J Invest Dermatol 2009;129:2480–8.
[88] Takato T, Iwata K, Murakami C, Wada Y, Sakane F. Chronic administration of myristic acid improves hyperglycaemia in the Nagoya–Shibata–Yasuda mouse model of congenital type 2 diabetes. Diabetologia 2017;60:2076–83.
[89] Pouillart PR. Role of butyric acid and its derivatives in the treatment of colorectal cancer and hemoglobinopathies. Life Sci 1998;63:1739–60.
[90] Lu N, Li M, Lei H, Jiang X, Tu W, Lu Y, et al. Butyric acid regulates progesterone and estradiol secretion via cAMP signaling pathway in porcine granulosa cells. J Steroid Biochem Mol Biol 2017;172:89–97.
[91] Bergsson G, Arnfinnsson J, Steingrímsson Ó, Thormar H. In vitro killing of Candida albicans by fatty acids and monoglycerides. Antimicrob Agent Chemother 2001;45:3209–12.
[92] Venugopal E, Ramadoss G, Krishnan K, Eranezhath SS, Bhattacharyya A, Rajendran S. Stimulation of human osteoblast cells (MG63) proliferation using decanoic acid and isopropyl amine fractions of Wattakaka volubilis leaves. J Pharm Pharmacol 2017;69:1578–91.
[93] Kaplan M, Koparan M, Sari A, Ozturk S, Kaplan SK, Erol FS. Can behenic acid (C22: 0) levels be a prognostic factor in glial tumors? Can J Neurol Sci 2013;40:854–6.
[94] Connell DL, Gradwell AJ. Fluid loss control additives for oil-based well-working fluids. Google Patents 1987.
[95] O'brien RD. Fats and oils: formulating and processing for applications. Boca Raton, FL: CRC Press; 2008.
[96] Jung S, Lee S, Lee H, Yoon J, Lee E. Oleic acid-embedded nanoliposome as a selective tumoricidal agent. Colloids Surf B Biointerfaces 2016;146:585–9.
[97] Golfetto I, Crawford M, Campo R. Treatment of hemorrhagic stroke with arachidonic acid. Nutr Neurosci 2001;4:75–9.
[98] Sethi P, Akintoye E, Thompson P, Mozaffarian D, Harris W. Higher plasma omega 3 fatty acid levels are associated with reduced risk for bleeding after cardiac surgery. J Am Coll Cardiol 2018;71:A1785.
[99] Stonehouse W, Conlon CA, Podd J, Hill SR, Minihane AM, Haskell C, et al. DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. Am Clin Nutr 2013;97:1134–43.
[100] Sanders TA. The role of fats in human diet. London, UK: Academic Press; 2016.
[101] He C, Qu X, Cui L, Wang J, Kang JX. Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. Proc Natl Acad Sci U S A 2009;106:11370–5.
[102] Heiskanen KM, Savolainen KM. Erucic acid and erucic acid anilide-induced oxidative burst in human polymorphonuclear leukocytes. Free Radic Res 1997;27:477–85.
[103] Asano J-i, Suzuki Y, Yajima S, Inoue K, Shimozawa N, Kondo N, et al. Effects of erucic acid therapy on Japanese patients with X-linked adrenoleukodystrophy. Brain Develop 1994;16:454–8.
[104] Kinoshita Y, Yoshizawa K, Hamazaki K, Emoto Y, Yuri T, Yuki M, et al. Mead acid inhibits the growth of KPL-1 human breast cancer cells in vitro and in vivo. Oncol Rep 2014;32:1385–94.
[105] Hammarström S. Conversion of 5, 8, 11-eicosatrienoic acid to leukotrienes C3 and D3. J Biol Chem 1981;256:2275–9.
[106] Whelan J. Dietary stearidonic acid is a long chain (n-3) polyunsaturated fatty acid with potential health benefits. J Nutr 2009;139:5–10.
[107] Hernandez E, RUSLI M, Griffin M. Phospholipid compositions enriched for palmitoleic, myristoleic or lauroleic acid, their preparation and their use in treating metabolic and cardiovascular disease. Google Patents 2013.
[108] Yang Z-H, Takeo J, Katayama M. Oral administration of omega-7 palmitoleic acid induces satiety and the release of appetite-related hormones in male rats. Appetite 2013;65:1–7.
[109] Field CJ, Blewett HH, Proctor S, Vine D. Human health benefits of vaccenic acid. Applied Physiology, Nutrition, and Metabolism 2009;34:979–91.