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An Overview about Versatile Molecule Capsaicin

R Arora, N S Gill, G Chauhan*, A C Rana

Rayat Institute of Pharmacy, Railmajra, District Nawanshaher, Punjab, India

ABSTRACT
Capsaicin is a pungent highly domesticated fat soluble alkaloid having its origin from Bolvia and Brazil. It is biosynthesized via phenylpropanoid pathway and branched chain fatty acid pathway. Mostly found in *trans* isomeric form. Its pungent analogues are synthesized using amidation reactions catalyzed by lipases and non-pungent analogues using acyl chain lengths and different substituent in aromatic ring. It acts by binding TRPV1 and shows wide applications in relief from pain, breast and prostate cancer, obesity, heart diseases and hair loses. Its increased intake may cause lesions of liver and kidney along with gastric cancer. A review is made of capsaicin research focusing mainly on its origin, biosynthesis, chemistry and pharmacology, including its toxicological aspects.

Keywords: Capsaicin, origin, biosynthesis, chemistry, pharmacology.

INTRODUCTION

Capsaicin **(1)** is the active ingredient of hot chili pepper, which is not only used as spice to foods, but can cause the body to heat up, promoting expenditure of calories. [1] Chemically it is a fat soluble phenolic compound which imparts pungent taste even if it is diluted to one part in eleven million parts of water. [2]

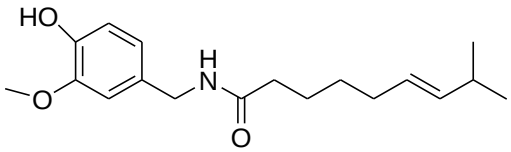

Fig. 1: Structure of Capsaicin

It is known with various synonyms, i.e. *N*-[(4-Hydroxy-3-methoxybenzyl]-8-methyl-*trans*-6-nonenamide, *N*-[(4-Hydroxy-3-methoxy-phenyl)methyl]-8-methyl-*trans*-6-nonenamide, *N*-(3-Methoxy-4-hydroxybenzyl)-8-methyl-non-*trans*-6-enamide, *trans*-8-methyl-*N*-vanillyl-6-nonenamide, Isodecenoic acid vanillylamide and 8-Methylnon-6-enoyl-4-hydroxy-3-methoxybenzylamide. [3] Capsaicin is a decylenic acid amide of vanillyl-amine. On variation in the acid portion of the molecule, different degree of pungency of analogues has been observed. [4]

Origin of capsaicin

For thousands of years spices play a major role in various food preparations to strengthen the taste. Among various spices, the fruit of *Capsicum*, (Hot chili peppers) is the mostly used spice. [5] Capsaicin originated in Bolivia and parts of Brazil and has been domesticated for at least 7,000 years. [6-7] There is archaeological evidence at sites located in southwestern Ecuador that chili peppers were already well domesticated more than 6000 years ago, and is one of the first cultivated crops in the America. [1] Chili peppers are mainly consumed as food additives in many regions of the globe because of their unique pungency, aroma, and color. [8] Indeed, a quarter of the world's population consumes hot pepper in some form daily. [9] The centre of diversity for *Capsicum* is in south-central South America with the majority of species having some range in Brazil and/or Bolivia. Some of the non-domesticated species are gathered for occasional use. [10-14]

Capsaicin, a major alkaloid among capsaicinoids produced only in *Capsicum* fruits. [15, 12] The genus *Capsicum* (family *solanaceae)* comprises of over 200 varieties ranging from the very hot habanero to the sweet bell peppers. These varieties are classified as "hot" or "sweet" based on Scoville "Heat" Units (SHU) as shown in Table 1. The hotter the pepper is, the higher the SHU value. [16]

Distribution of capsaicin in the fruit of the plant

Recent studies indicate that capsaicin is mostly located in vesicles or vacuole like sub-cellular organelles of epidermal cells of placenta in the pod. [17] The highest concentrations of capsaicin are found in the ovary and in the lower flesh (tip) and the lowest content of capsaicin can be found in the seeds. [18] The gland on the placenta of the fruit produces capsaicinoids. [19] The seeds are not the source of pungency but they occasionally absorb capsaicin because they are in close proximity to the placenta. No other plant part produces capsaicinoids. The majority, about 89%, of the capsaicin is associated with the placental partition of the fruit and nearly

author : Mr. Gaurav , Pharmaceutical Chemistry Laboratory, Rayat Institute of Pharmacy, Railmajra, District Nawanshaher, Punjab, India; **Tel.:** +91-9996266655; **E-mail:** chauhan971@gmail.com

5-6% in the pericarp and the seed. [20] Composition of capsaicin may vary among different varieties of same species and with fruit of a single variety. The pungency is influenced with the weather conditions such as heat wave and it increases with the growth of the maturity of fruit. [21]

Table 1: Scoville "Heat" Units (SHU) for variety of peppers

Pepper varieties	Scoville "Heat'' units
Habanero	200,000 - 300,000
Bird's Eye	100,000-125,000
Carolina Cayenne	100,000-105,000
Thai	70,000 - 80,000
Red Chili	50,000 - 60,000
Tabasco	30,000 - 50,000
Chili Pequin	30,000 - 40,000
Cayenne	35,000 - 40,000
Chile de Arbol	15,000-30,000
Jalapeno	7,000 - 25,000
Hidalgo	6,000-17,000
Yellow Wax	15,000-17,000
Santa Fe Grande	5,000 - 6,000
Jalapeno (another variety)	3,000 - 4,000
Ancho	2,500 - 3,000
Anaheim	1,000-1,500
Bell peppers	less than 200

Properties of capsaicin

Capsaicin ($C_{18}H_{27}NO_3$) is an odorless white crystal (monoclinic, rectangular plates, scales in petroleum ether) with severe burning pungency. One part in 100,000 can be detected by tasting. It has a molecular weight of 305.4118 g/mol, melting point of 65°C, boiling point at 0.01 mm Hg is 210-220°C, sublimate at 115°C, UV_{max} at 227, 281 nm (€ = 7000, 2500), slightly soluble in carbon disulfide, hot water, practically insoluble in water, freely soluble in alcohol, ether, benzene and chloroform It is fairly resistant to acids and alkali solutions at room temperatures. [16]

Chemistry of capsaicin

Capsaicin molecular structure has been first resolved by Nelson and Dawson in 1919. [22] Since the double bond seems to prevent the internal rotation, thus capsaicin shows *cis/trans* isomerism. But mostly it is found in *trans* isomeric form because in *cis* form the $–CH(CH_3)_2$ and the longer chain on other side of the double bond will be close together causing steric hindrance due to slight repulsion between them (Fig. 2). [23]

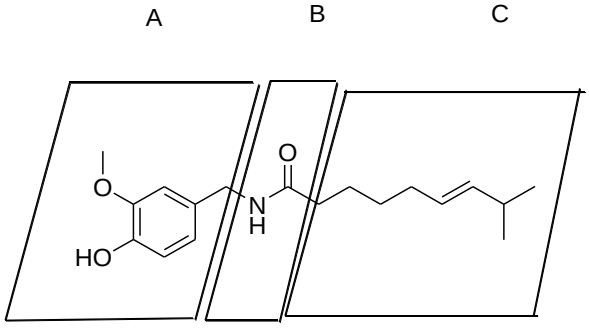

Fig. 2: Regions of the molecule of capsaicin: A-aromatic ring, B-amide bond, and C-hydrophobic side chain

Structure Activity Relationship (SAR)

SAR studies can be rationalized by dividing capsaicin molecule into three regions: A (aromatic part), B (amide bond) and C (hydrophobic side chain). For potent agonist activity, substituent at 3 and 4 positions in the aromatic ring is necessary, and the phenol 4-OH group in capsaicin analogue is of particular importance, H-bond donor/acceptor properties of the phenol group are important for the agonist activity. [24] C-Region in structure (hydrophobic side chain) e.g. an octyl chain and substituted benzyl or group, is required for high potency. Optimally, such aralkyl groups are substituted in the *para* position by small hydrophobic moieties. [25] It was reported that lateral chain lengths are important for the bioactivity of capsaicinoids, which was higher between 8 and 9 carbons atoms. [26]

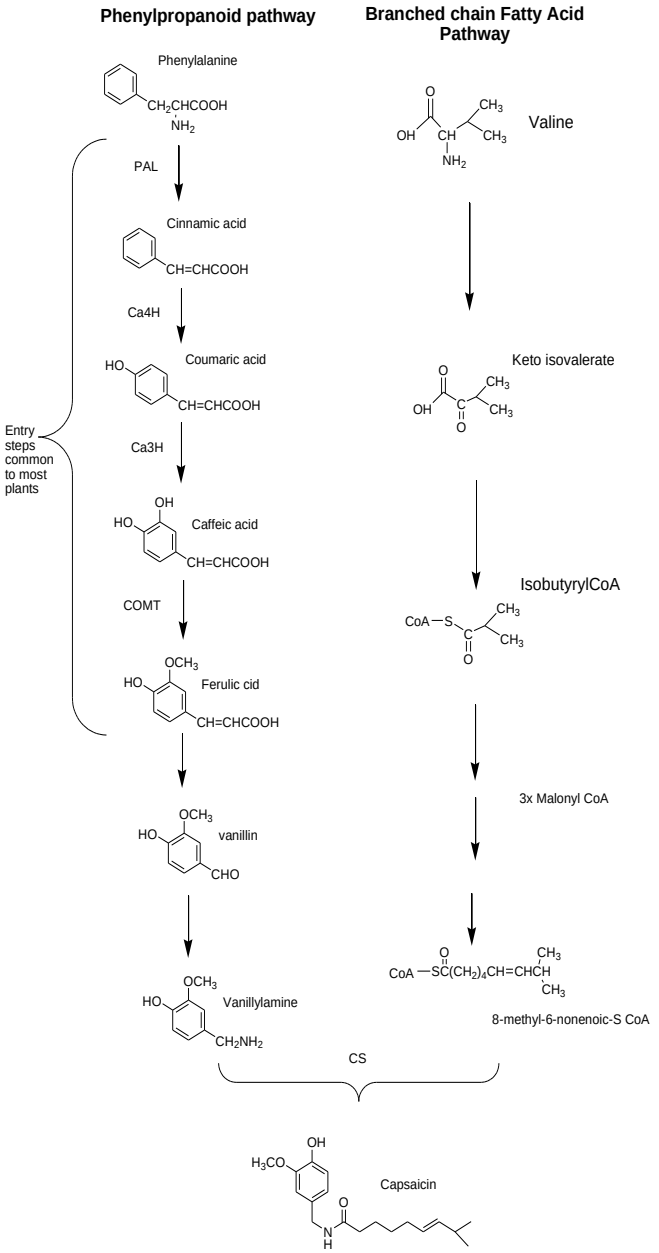

Fig. 3: Proposed pathway for biosynthesis of capsaicinoids. The enzymes indicated on the pathway are: phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (Ca4H), coumaric acid 3-hydroxylase (Ca3H), caffeic acid *O*-methyltransferase (COMT), and capsaicinoid synthetase (CS)

Genetic regulation of capsaicin synthesis

Chili genotypes exhibit wide variation in capsaicin accumulation in response to genetic and environmental factors. [27] It is revealed through the genetic analysis of capsaicin accumulation done by molecular mapping that the presence of a Quantitative Trait Locus (QTL) called *Cap* contributes to an increasing pungency level. [28] Most of the genes are involved in the biosynthesis of capsaicin but little is known about the location and action of the specific gene controlling capsaicin accumulation. Transcript accumulation

has been reported for the genes *Pal, Ca4h, Comt*, corresponding to the phenylpropanoid pathway and the genes coding for enzymes i.e. *Kas, Acl and Fat* are involved in fatty acid metabolism [29-30] Spiciness is known to be inherited as a dominant trait and is associated with the *Pun1* locus. AT3 is the product of *Pun1* locus which corresponds to a putative acyltransferase, and specific to the placenta in pungent genotypes. *Pun1* exhibits a dominant behavior which determines pungent genotype occurrence. Initially it was assumed that AT3 could correspond to CS. However, the *csy1* gene was isolated and found to code for CS and its sequence differs from AT3, indicating that *Pun1* does not codify for CS. [31, 15, 7] It was thus established that the product of *Pun1* is related to development of the vesicles where capsaicinoids accumulate and not to their production, although formation of these vesicles is vital for pungent phenotype occurrence. [32]

Biosynthesis of capsaicin

The condensation of vanillylamine with a short chain branched fatty acid results in the synthesis of capsaicinoids. A possible biosynthetic pathway is shown in (Fig. 3).

The production of vanillylamine is *via* phenylpropanoid pathway and the branched chain fatty acid is synthesized from a branched-chain amino acid, e.g. valine. Various evidences in support of this pathway include radiotracer studies, determination of enzyme activities, and the abundance of intermediates as a function of fruit development. [33-38]

Chemical and enzymatic synthesis

Research efforts have been applied to synthesize various synthetic analogues of capsaicin which have properties similar to natural types. [39] Enzyme catalyzed synthesis is advantageous over chemical synthesis in that it involves use of non-toxic reagents and substrate specificity. [40] A number of studies have been done on synthesis of capsaicin analogues using amidation reactions catalyzed by various lipases. [41] In these trials, vanillylamine condensed with fatty acid derivatives, used as a substrate in the oleose phase, resulted in 40-59% capsaicin yields and synthesis of various analogues at 2-44% yields and with from 4-18 carbons. [42] Various non-pungent analogues were synthesized using different acyl chain lengths and chemical substitutes in the aromatic ring. They obtained two pungent analogues and other very low pungency analogues with potential uses (Fig. 4). [43-44] Capsaicin and its analogs are produced industrially using chlorinated fatty acids and amines at temperatures between 140°C and 170°C under moderate pressure. [45] Use of bioisosterism has been reported by Choi and Yoon to synthesize 1-hydroxy-2-pyridone, a capsaicin analogue with similarity in its activity. [46] A study using chemo selective esterification of phenolic acids with alcohols for vanillyl nonanoate synthesis involved reactions between vanillyl alcohol and nonanoic acid using tetrahydrofurane as reaction medium and equimolar amounts of diisopropyl azodicarboxylate and triphenyl phosphine. After running for 24 h at room temperature, the reaction produced a 67% vanillyl nonanoate yield. [47]

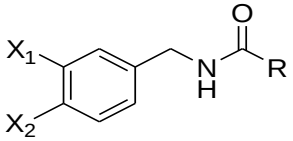

Fig. 4: Various Capsaicin analogues synthesized using different amines and donors as substrates

Compound	X_1	X_2	Compound	R
1	MeO-	OH-	A	- C_3H_7
2	H-	H-	B	- C_5H_{11}
3	MeO-	H-	C	- C_7H_{15}
			D	- C_9H_{19}
			E	- $C_{11}H_{23}$
			F	- $C_{13}H_{27}$
			G	- $C_{15}H_{31}$
			H	- $C(CH_2)_7CHCH(CH_2)_7CH_3$

In another study, cerium chlorate (III) was used as a reaction catalyst in selective esterification of phenolic alcohols, resulting in a 70% vanillyl nonanoate yield. [48] Due to toxicity of the required reagent, chemical synthesis of capsaicin has limited success over enzymatic synthesis. Capsinoids (capsiate, dihydrocapsiate and nordihydrocapsiate), other analogues of capsaicin have close structural resemblance with capsaicinoids except for their center linkage: amide moiety in capsaicinoids and an ester moiety in capsinoids (Fig. 5). Bio-potency of capsinoids is similar to capsaicin without the pungency or sensory irritation. Though interest of some groups has been focused in these molecules but their mechanism of action are poorly understood. [49, 39] They are naturally found in few species, thus are synthesized chemically but their chemical synthesis is also complicated due to the presence of terminal methyl group and double bond. [50]

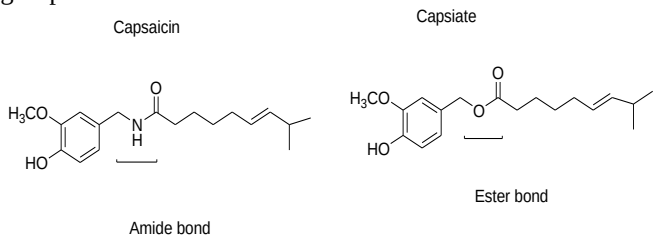

Fig. 5: Structures of capsaicin and capsiate

***In-vitro* synthesis of capsaicin**

In vitro synthesis is another good alternative for synthesis of capsaicin and its analogues. Capsaicinoid production via cell or tissue culture can be augmented be addition of biosynthetic pathway precursors and intermediaries as phenylalanine, ferulic acid and vanillylamine showing encouraging results. [51] Cell suspensions are used to study the various phenyl propanoids including phenylalanine. In this study the addition of 100 µM of either phenylalanine, cinnamic acid or caffeic acids did not cause significant increment in capsaicinoids content during the growth cycle. But the addition of 100 µM of vanillin, vanillylamine, *p*-coumaric acid and ferulic acid did increase 10, 7.5, 5.2 and 2.5 fold higher the capsaicin production as compared to control, respectively. [52] Coumaric acid and elicitors such as phycocyanin, synapinic acid, salicylic acid and curdlan result in different capsaicin production levels. [53] It has been reported that administration of the supplements L-ascorbic acid and D-limonene in the culture medium lead to three fold increases in the production of capsaicin and with the use of *p*-fluorophenylalanine resistant callus culture, capsaicin production increases to 45%. [54]

It has been found that immobilized cell culture shows more efficient *in vitro* production of capsaicin then the cell suspension culture because the former use the precursors for capsaicin synthesis and latter use them for primary metabolism. [55] Since the placenta contains the biochemical machinery needed for capsaicin synthesis thus the placental tissue cultures, with higher capsaicin production in

immobilized versus suspension cultures. Finally, the use of elicitors phycocyanin (0.25%), sinapic acid (0.05 mM), salicylic acid (2 mM) and curdlan (0.0625%), increases capsaicin upto 8.9 fold in the cell suspension cultures. Although salicylic acid and curdlan had not effect, curdlan in combination with tyrosine, increases the accumulation of capsaicin 8.7 fold, and had an effect in the phenylalanine ammonialyase activity. [56, 53] *In vitro* culture is clearly a promising alternative for capsaicin production since it allows for control of culture conditions and is scalable.

Pharmacology of capsaicin

Pharmacokinetics

Capsaicin is found to be absorbed effectively and reach maximum concentration if it is applied topically. Half life of the capsaicin is 24 h. [57] In a study it was found that orally administered capsaicin was absorbed and reaches maximum concentration in blood within 1h after administration and then diminished notably until being undetected after 4 days. [58] During metabolism of capsaicin three major metabolites have been identified as 16-hydroxycapsaicin, 17-hydroxycapsaicin and 16, 17-dihydrocapsaicin. [59] *In vitro* studies on human skin suggest that most capsaicin remained unchanged while a small fraction was metabolized to vanillylamine and vanillyl acid which further suggested role of cytochrome P_{450} enzymes in transformation capsaicin in human skin. [60, 58]

Mechanism of action

Capsaicin acts by binding to transient receptor potential vanilloid 1 (TRPV1), previously known as the vanilloid receptor, which is mainly expressed in the sensory neurons. [61] This receptor is located primarily in the small fibers of nociceptive neurons. It is non selective in nature and is ligand operated cationic channel. TRPV1 is also broadly distributed in tissues of the brain, bladder, kidneys, intestines, keratinocytes of epidermis, glial cells, liver, and polymorphonuclear granulocytes, mast cells, and macrophages. [61-62] TRPV1 contains 838 amino acids and has a molecular weight of 95 kDa in humans, consisting of six transmembrane domains with a short pore-forming region between the fifth and sixth transmembrane domains. [63] It regulates intracellular calcium levels by coupling with a non-specific cation channel permeable to sodium and calcium ions, and is located in the plasma membrane and the endoplasmic reticulum. [64-65] Endogenous substance like endovanilloids can regulate and activate this channel and diverse exogenous stimuli which includes chemical agonists as capsaicin, olvanil and resiniferatoxin, ligands highly lipophilic that share structural similarity to several endogenous fatty acids identified as TRPV1 agonists. [66] Compounds that are used as antagonist for TRPV1, includes capsazepine, iodoresiniferatoxin, ruthenium red, A-425619, SB-366791, AMG9810 and SB-705498. [67] A heat sensitive subunit of TRPV1 is responsible for burning sensation caused by capsaicin. When capsaicin binds to TRPV1, there is an increase in intracellular calcium which triggers the release of neuropeptides such as substance P and the calcium gene-related peptide (CGRP). Binding between capsaicin and sensory neurons produces pain, inflammation and a localized heat sensation. When applied topically, it desensitizes the sensory neurons skin due to depletion of substance P thus causing analgesic action. [68] A number of studies include results showing that it participates in release of somatostatine, CGRP and endotheline. [69-73]

Clinical uses of capsaicin

Pain relief

Capsaicin helps in reducing inflammatory heat and noxious chemical hyperalgesia and pain from rheumatoid arthritis or fibromyalgia. [74] Capsaicin is also the key ingredient in Adlea, a drug which is in Phase 2 trial as a long-acting analgesic to treat post-surgical and osteoarthritis pain. [75] Capsaicin is also an ingredient of some over the counter pain reliever creams at a concentration of 0.075% or lowers. [76]

Cancer prevention

Anticancer activity of capsaicin has been reported for a long time. [77] In the cultured cells, capsaicin was able to block breast cancer cell migration and kill prostate cancer cells, and dihydrocapsaicin was reported to induce the autophagy in HCT116 human colon cancer cells. [78-80] Natural capsaicin also shows inhibition of the growth of leukemic cells. [81] Cellular proliferation plays a major role in multistage carcinogenesis and is a critical hallmark for cancer prevention. Capsaicin represses the growth of various immortalized or malignant cell lines via induction of cycle arrest, apoptosis, autophagy, and/or via the inhibition of cellular metabolic activation. [82-85, 78-79] Capsaicin is found to inhibit isoform of enzyme cytochrome P450 involved in the detoxification of many low-molecular-weight carcinogens. [86] Capsaicin only selectively inhibits the growth or induces apoptosis of immortalized or malignant cell lines, but not of normal cell lines. [87] Studies reveal that the metabolites of capsaicin (such as the reactive phenoxy radicals) may attack the DNA and trigger the mutagenicity and malignant transformation. [88]

Weight reduction

As obesity is posing a great threat to human health, thus various strategies for weight loss and maintenance are gaining great attention worldwide. Since energy metabolism along with thermogenesis play an important role in the regulation of obesity, chili peppers, therefore, are thought as the potential foods having anti-obesity properties. [89-91] Capsaicin enhances energy expenditure and reduces body fat accumulation in animal experiments as well as clinical studies. [92-93] Molecular mechanisms responsible for the anti-obesity effect of capsaicin showed that thermogenesis and lipid metabolism related proteins were markedly altered upon capsaicin treatment, which shows the important role of capsaicin in regulating energy metabolism. Although capsaicin has anti-obesity effects, the potential side effects limit its application in clinic. [90] The non-pungent CH-19 sweet pepper (the major source of natural capsinoids), showed an attractive option for weight loss. It has been shown that a single dose of CH-19 sweet pepper could increase the body temperature and oxygen consumption while repeated CH-19 sweet pepper intake could reduce the body weight and promote the fat oxidation. [92]

Cardiovascular benefits

There is evidence that capsaicinoids have potential beneficial effects on the cardiovascular system to treat various cardiovascular threats in human beings that include coronary heart disease, myocardial infarction, hypertension and atherosclerosis. [94-95] Cardiovascular system contains capsaicin-sensitive sensory nerves, which play an extensive role in regulating cardiovascular function through the release of multiple neurotransmitters such as CGRP through activating TRPV and Substance P. [95-96] Indeed, acute application of capsaicin can mimic the ischemic

preconditioning like cardiac protection, which was blocked by CGRP-(8-37), the selective CGRP receptor antagonist. [97] Capsaicin has been reported to inhibit platelet aggregation and the activity of clotting factors VIII and IX a property which in the reduction of the incidence of cardiovascular diseases. It has been suggested that capsaicin was able to pass through plasma membrane of platelets and alter membrane fluidity. [98-99] Recent studies showed that due to presence of TRPV1 in human platelets. [100] Capsaicin induces Ca^{2+} release from intracellular platelet stores and subsequently contributed to ADP and thrombin induced platelet activation. The oxidation of low density lipoprotein (LDL) leads to development and progression of atherosclerosis. *In vitro* it has been reported that capsaicin was able to increase the resistance of LDL to oxidation by delaying the initiation of oxidation and/or slowing the rate of oxidation. Regular consumption of chili for 4 weeks has been found to increase the resistance of serum lipoproteins to oxidation in adult men and women. [101] This shows that due to the antioxidant property of capsaicinoids and they act as potential clinical value on the prevention of cardiovascular diseases, such as atherosclerosis and coronary heart disease in particular.

Gastrointestinal benefits

Capsaicin-sensitive sensory nerves are also present in gastrointestinal system which plays a crucial role in maintenance of gastrointestinal mucosa integrity against injurious interventions. It has been reported that capsaicinoids exert either beneficial or detrimental effects on gastrointestinal mucosa depending on the dose and/or duration of drug treatment. A high dose of capsaicinoids usually led to the exhaust of neurotransmitters and the damage of capsaicin-sensitive sensory nerves, which might have detrimental effects on the gastrointestinal systems. [102, 95] However, a low dose of capsaicinoids could increase the basal gastric mucosal blood flow and gastric mucus secretion, and facilitate gastric epithelial restitution, which were beneficial to gastrointestinal defense. [103]

Other utilities

The use of capsaicin in treatment of burning mouth syndrome has been reported in many literatures but its use has been restricted because of their adverse reactions. [104] Capsaicin by activating vanilloid receptor-1, increases the release of calcitonin gene-related peptide (CGRP) from sensory neurons, and CGRP has been shown to increase insulin-like growth factor-I (IGF-I) production which plays an important role in hair growth. Thus it has been reported that combined administration of capsaicin and isoflavone might increase IGF-I production in hair follicles, thereby promoting hair growth. [105]

Toxicological aspects of capsaicin

Capsaicin has been reported to cause histopathological and biochemical changes including erosion of gastric mucosa and lesions of liver and kidneys, after oral ingestion. Experiments on subchronic and chronic toxicity of orally applied preparations reflect the local irritative effect of capsaicin. In another experiment the carcinogenicity of the capsaicin has been studied and it was found that it did not show carcinogenicity effect in mice at a dose up to 375 mg capsaicinoids/kg bw/day. In studies, in human, the increased intake of chilies in Mexico with a daily intake of 200mg capsaicinoids/person showed increased risk of gastric cancer. Based on rough assumption of low toxicity for oral doses of 4mg capsaicinoids/kg bw in humans and using a safety factor of 20, a TMDI expressed as total capsaicinoids was set at 0.2 mg/kg bw. [106, 3]

Capsaicin has been widely investigated, and is an important molecule in area of research in medicinal field but their clinical applications are still very limited due its low selectivity and high toxicity. Further pungency also limits its use in clinical trials. As an agonist of TRPV1, the precise mechanisms for the interaction between capsaicin and TRPV1 are not well understood. But this is a promising molecule if some modifications in the chemical structure of capsaicinoids are done to overcome its poor properties and drawbacks. Various non-pungent analog molecules have been developed in response. Therefore, future studies will remain focused on exploiting this molecule for medicinal utilities.

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