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Bioactivities of Allicin and Related Organosulfur Compounds from Garlic: Overview of the Literature Since 2010

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THE medical values of garlic, a traditionally used allium vegetable, were recognized as early as 3000 BC. Allicin, one of the major organosulfur compounds present in garlic, is associated with a wide variety of benificial activities like anticancer, anti-inflammatory, antiarthritic, antimicrobial, anti-diabetic, cholesterol-lowering, the potential to lower cardiovascular disorders, ameliorating neuron functions, etc. In this article, we have reviewed biological activities of allicin and related organosulfur compounds since the year 2010, using various scientific websites like Pub med, Google Scholar, Science Direct etc. Our literature review has highlighted many organo sulphur components of garlic and their possible mechanisms in curing different diseases. The information provided in this review will provide strategies for efficient organosulfur-based treatments of several diseases in future. Further research efforts are needed for a clear understanding of the interconnection between these functional components and various chronic diseases.

Keywords: Allicin, Organosulfur compounds, Garlic, Pharmacology, Clinical trials.

Introduction

In recent days, due to high efficacy and low adverse effects, natural products have attracted the drug inventors as useful therapeutic agents for the treatment of various diseases [1]. Allium vegetables, such as garlic (*Allium sativum*) is used in many countries throughout the world as medicine since ancient days for the therapeutic efficacies, as a result of organosulphur compounds, present in it. Garlic contains volatile oil and approximately 33 sulfur compounds like allicin, alliin, ajoene, diallyl disulfide (DADS), diallyl trisulfide (DATS), S-allyl-L-cysteine (SAC), vinyldithiin, S-allyl mercaptocysteine (SAMC), etc [2]. Allicin is the major and most biologically active organosulphur compound in garlic, isolated and identified in 1944. Allicin is poorly miscible in water, which has a characteristic odour like freshly crushed garlic [3]. It is readily obtained from alliin by the enzymatic activity of alliinase (Fig. 1). Alliin, an oxygenated sulphur amino acid present in the garlic clove, is the stable precursor of allicin [4].

In spite of enough scientific evidence all

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through the past decade concerning the biological effects of allicin and related organosulfur compounds, there are lacks of updated reviews on them. In this article, we have reviewed biological activities of allicin and related organosulfur compounds from the year 2010, using various scientific websites like Pubmed, Google Scholar and Science Direct etc. Our aim is to focus the recent scientific evidence in our review paper, trying to find the right mechanisms of these compounds for various diseases. Moreover, we have discussed some chemistry and clinical trial study findings of allicin to provide a complete picture.

Clinical Trials

A clinical trial was executed on allicin for investigating its effectiveness and safety profile, in stage II oral submucous fibrosis (OSF). Some quality of life such as mouth opening, burning sensation, and oral health were improved in the patients with allicin showing its potentiality as an adjunctive therapeutic drug [5]. Another clinical trial was carried out on allicin to evaluate the efficacy and safety profile of topical application of allicin oral adhesive tablets in minor recurrent aphthous ulcerations (MiRAU). This clinical study showed that these tablets reduced ulcer size and removed ulcer pain of the without significant side effects [6]. In another clinical study allicin was orally administered for the treatment of Behcet's disease (BD), occurs due to oxidative stress (OS) aggression, in patients with mucocutaneous lesions**.** Allicin significantly improved OS-related parameters by inhibiting OS and regulating oxidant/antioxidant status balance. Moreover, allicin was found to be safe and was effective in the treatment of BD [7]. In a previously reported clinical trial, a high dose (180 mg/day) of allicin showed effectiveness to prevent the common cold [8].

Biological Activities

Anticancer & antitumor activity

Though the exact pathway by which allicin affects the growth of cancer cell lines is still not clear, its anticancer activity has been reported through various studies. Allicin acted as a novel anti-oesophageal cancer agent for oesophageal squamous cell carcinoma (ESCC). Investigators reported that cell viability and invasion ability in ESCC cells were considerably alleviated after allicin therapy. Allicin was seen to reduce the cell population in the G0/G1 phase and persuade G2/M phase arrest [9].

Allicin has been reported to show the hepatoprotective effect and antitumor activity. Researchers demonstrated through the *in vivo* experiment that combined therapy of allicin and 5-fluorouracil (5-FU) exhibited an outstanding inhibitory effect on the growth of hepatocellular carcinoma (HCC) in mice. Allicin increased 5-FU induced cytotoxicity in HCC cells. Allicin sensitized HCC cells to 5-FU persuaded apoptosis through ROS (Reactive oxygenated species)-mediated mitochondrial pathway. Therefore, allicin may be a perfect booster to the chemotherapy regimen of HCC [10].

Allicin ameliorated the apoptotic death of colon cancer cells mediated by NF-E2-related factor-2 (Nrf2). The *in vitro* studies suggested that allicin affected the multiplication of colon cancer cell lines HCT-116, LS174T, HT-29, and Caco-2. HCT-116 apoptotic cell death took place after administering allicin through enhancing hypodiploid DNA level, decreasing counts of B-cell non-Hodgkin lymphoma-2 (Bcl-2), increasing amounts of Bcl-2-associated X protein (BAX) and the increasing ability to liberate Cytochrome-C from mitochondria [4].

Allicin was seen to be chemopreventive to gastric cancer. It decreased the growth of cancer cells at G2/M phase via the caspase-dependent/-independent pathway. The probable mechanism may be enzymatic activity modulation [11].

A study was performed to investigate the effects of allicin in buccal pouch carcinogens is induced by 7, 12-dimethylbenz(a) anthracene (DMBA). The experiment showed that allicin remarkably reduced tumour volume and tumour load. Allicin treatment was observed to normalise DMBA altered glycoconjugates in plasma as well as buccal mucosa tumours. Researchers concluded that the allicin has appreciable power to safeguard and reinstate the cell surface glycoconjugates during oral carcinogenesis [12].

Anti-inflammatory & anti-arthritic activity:

Inflammation has long been a well-known symptom of many diseases, including arthritis. Arthritis is a major problem in the people throughout the world, which affects the joints and connective tissues like bones, muscles, cartilage, and tendon [13]. In arthritis, cyclooxygenase-2

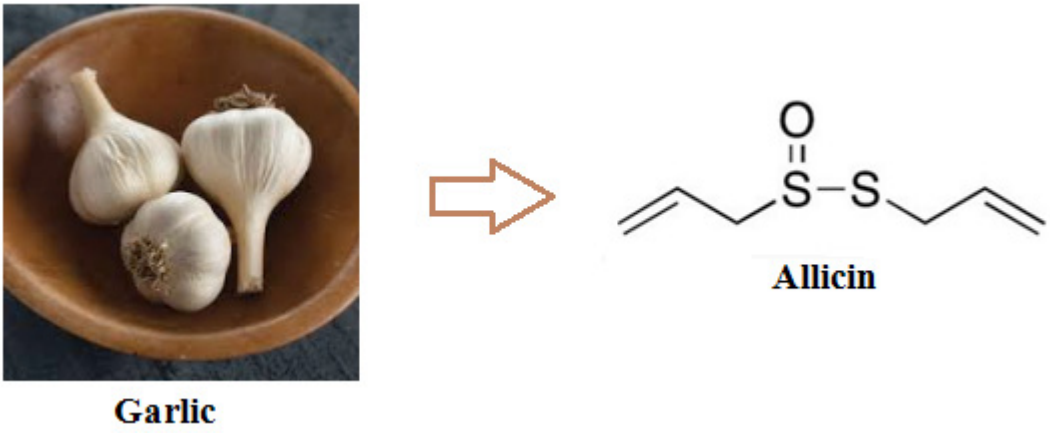


Fig. 1. Allicin from garlic

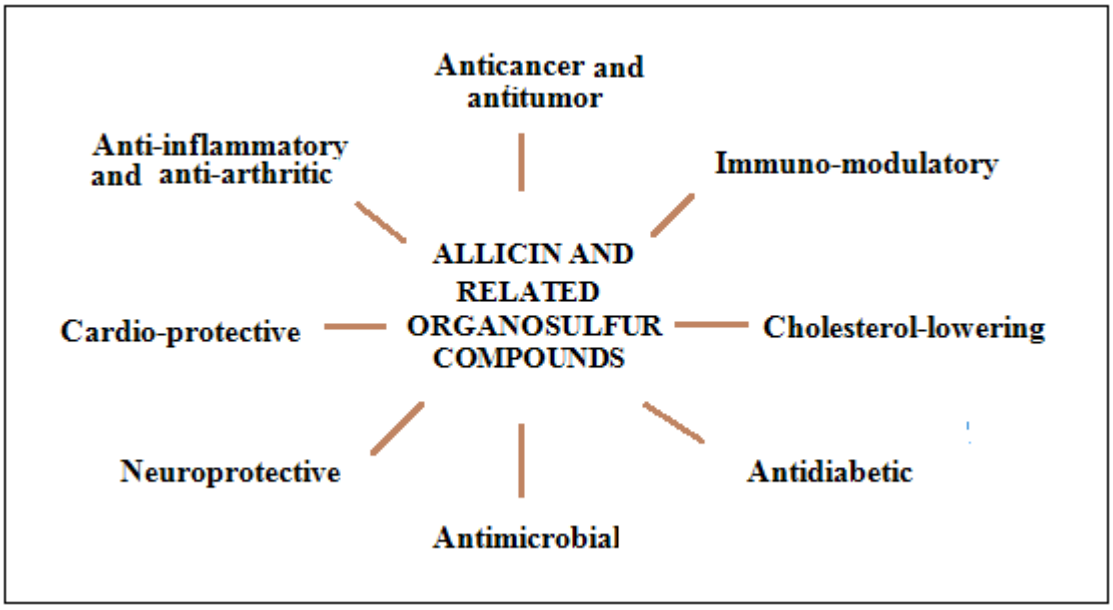


Fig. 2. Different biological activities of allicin and related organosulfur compounds from garlic

(COX-2) selective inhibitors are very much effective. Most of the currently available COX-2 inhibitors are sulphur-containing molecules [14,15] allicin is one of them. Very recently a study has shown that allicin is a significant COX-2 inhibitor, in a dose-dependent manner [16]. Some other sulfur compounds of from garlic such as (Z, E)-ajoene and its three sulfonyl analogs were also tested for their anti-inflammatory properties. These compounds showed their anti-inflammatory potential by suppressing the LPS-induced production of nitric oxide (NO) and prostaglandin E2 (PGE2) in macrophages. Additionally, they hindered the expression of NO synthase (iNOS) and cyclooxygenase-2 (COX-2) genes in lipopolysaccharide (LPS)-activated macrophages [17].

Allicin treatment is observed to be effective for all type of rheumatic and catarrhal conditions and has the most prominent activity in the treatment of rheumatoid arthritis [18]. In a study, the anti-inflammatory and antioxidant potency of allicin has been compared with piroxicam, a prominent anti-inflammatory drug. The study showed statistically significant anti-inflammatory efficacy of allicin, comparable to the piroxicam standard [13].

Allicin showed potential in the treatment of ankylosing spondylitis (AS) as an anti-inflammatory agent. Allicin markedly reduces AS perhaps at a dose of 200 mg/kg b. w., via alleviating the secretion of the inflammatory factors in mice. Moreover, Allicin considerably suppresses HLAB27 protein expression [19].

Allicin exhibited an appreciable anti-inflammatory effect in rat models of carrageenan-induced paw edema. A reduction in edema volume was observed, which was similar to that of diclofenac, a conventional anti-inflammatory drug [20]. Oral administration of allicin showed comparable activity with the standard drug diclofenac sodium [21]. Allicin and few other organosulpher compounds also suppressed LPS-induced inflammation [22].

Cardio-protective activity

Cardiovascular diseases are the most complex and cause of many deaths worldwide in recent days, which are around 17 million per annum according to the world health organization (WHO). The oxidation of low-density lipoprotein (LDL) causes atherosclerosis frequently. Allicin acts as an antioxidantat the physiological level in lower doses. Allicin regulates the expression of various anti-oxidative enzymes via Nrf2/Keap1 binding [23].

Allicin is considered as a cardioprotective agent and showed effects on vascular oxidative stress in cultured human umbilical vein endothelial cells (HUVECs) by activating Nrf2, which controls the defense against oxidative stress and inflammation [24].

The antihypertensive activity of allicin in dexamethasone-induced hypertension has been investigated. An experiment showed that systolic blood pressure (SBP) increased significantly due to dexamethasone treatment, while treatment with allicin markedly decreases the SBP. Allicin treatment also helps to recover dexamethasone-induced anorexia and weight loss. However, its antihypertensive mechanism is not fully clear, further studies needed to explore in detail [25].

Allicin was confirmed to show a protective effect in rat cardiomyoblasts (H9c2 cells) from hydrogen peroxide (H_2O_2)-induced oxidative injury. Due to the antioxidant properties of allicin, it can prevent the oxidative stress-induced injury, thus, become effective in cardiomyoblasts (H9c2 cells). The main mechanism is scavenging extracellular (H_2O_2) or free radicals. It was also believed that the mechanism of H9c2 cells protection by allicin may be through inhibiting intracellular reactive oxygen species (ROS) production instead of scavenging extracellular (H_2O_2) or free radicals. Additionally, allicin may be effective in ischemic condition by reducing free radical-induced myocardial cell death [26]. An experiment showed that allicin protected angiotensin II-induced cardiac hypertrophy. Angiotensin II infusion caused an increase in heart rate, blood pressure, and heart weight to body weight ratio, and as a consequence anatomical and functional changes occur, like increased left ventricular (LV) mass, LV end-diastolic diameter, and decreased fractional shortening. Allicin was effective in these cases and prevents cardiac hypertrophy by enhancing the Nrf2 (Nuclear factor-like 2) antioxidant signalling pathways. Allicin treatment also caused the accumulation of interstitial collagen and collagen I/III, decrease in the levels of reactive oxygen species, protein carbonyl and thiobarbituric acid reactive substances (TBARS), an increase in glutathione peroxidise (GPx) activities [27].

Allicin-treatment was fruitful in the myocardial infarction by improving cardiac function. Allicin was seen to reduce creatine kinase and lactate dehydrogenase amounts dose-dependently and it also significantly attenuated the myocardial apoptotic index and BAX expression. The probable mechanism of the cardioprotective effect of allicin was proposed to be obstruction of Bcl-2/BAX signaling pathway-dependent apoptosis and thereby amelioration of cardiac function [28].

In an experiment to determine the cardioprotective effect of allicin on the myocardial injury of rats, abnormalities like rising and fall of hemodynamic parameters were evidently maintained to normal levels in allicin treated animals. Allicin also prevented myocardial fibrosis in rats [29].

Neuroprotective Effect

The potency of allicin as a neuroprotective agent against traumatic brain injury (TBI) in rats had been investigated and it was found that allicin treatment promptly alleviated brain edema and apoptotic neuronal cell death in the injured cortex. Allicin exerted a protective effect against TBI through Akt/eNOS pathway in a dose-dependent manner [30]. Allicin also was used in ischemic stroke for its neuroprotective property [31].

S-allyl-L-cysteine from garlic considerably saved cultured rat hippocampal neurons (HPNs) against stress-induced neuronal cell death by inhibiting calpain, a Ca^{2+} dependent cysteine protease, via decreasing the level of intracellular Ca^{2+} [32]. Few synthetic derivatives of S-allyl-l-cysteine (SAC) also showed the same effect. Among them, *S*-propyl-l-cysteine (SPC) was the strongest one [33].

Diallyl disulfide showed the hippocampal neurogenesis and neurocognitive functions through regulating extracellular signal-regulated kinase (ERK) and brain-derived neurotrophic factor (BDNF)-cAMP response element binding (CREB) protein signalling [34]. Diallyl disulfide also exhibited neuroprotective effects in transgenic mice [35].

It was discovered that the motor functional recuperation and neuron destruction against spinal cord injury (SCI) was improved by allicin in rats due to its anti-oxidant and anti-inflammatory effects of it. Allicin showed its safeguard effect by mediating Nrf2 nuclear translocation [36].

Z-ajoene from garlic exerted neuroprotective effects by reducing lipid peroxidation and antioxidant or anti-inflammatory activities [37]. A few other clinically proven neuroprotective agents from garlic are *S*-propargylcysteine and *S*-methyl cysteine and N-α-(1-deoxy-D-fructos-1-yl)-L-arginine [38,39].

Antimicrobial activity

Allicin has antimicrobial potency against multi-drug resistant microorganisms, but the *in vivo* activity of allicin has not yet well documented in preclinical and clinical trials. Allicin showed evident antioxidant activity, and high membrane permeability which allows its fast penetrationinto the bacterial cell compartments [40]. Burkholderiacepacia complexes (Bcc) are the major bacterial phytopathogens for alliums and an intrinsically multiresistant and life-threatening pathogen for humans. Allicin was found to possess inhibitory activities against Bcc. The molecular mechanisms of allicin with a recombinant form of a thiol-dependent peroxiredoxin (BCP, Prx) from *B. cenocepacia* was also investigated. It was observed that allicin modifies an essential BCP catalytic cysteine residue. It suggests the role of allicin as a general electrophilic reagent to target protein thiols [41].

A comparison study to determine the potency of allicin with fluconazole was performed against *Candida albicans* infections in the mouse model. The efficacy of anticandidal effects of allicin was confirmed as an adjuvant therapy to fluconazole [42].

An investigation of the combination of allicin and silver nanoparticles (AgNPs) was performed against methicillin-resistant *Staphylococcus aureus* (MRSA) in an animal model. The study evidently suggested that allicin produces a synergistic activity when used in combination with AgNPs [43].

In a study, allicin exhibited antimicrobial activity against *P. mirabilis* by inhibiting urease activity. Relative urease activity in both prelysed and intact cells was inhibited by allicin in a concentration-dependent manner [44].

Antidiabetic activity

Researchers have seen the potential immunomodulatory effect of allicin in doses of 16 mg/kg i.p. when it was tested on type1 diabetic rats. Allicin treatment effectively reduced autoantibodies

TABLE 1. Major bioactive organosulphur compounds of garlic.

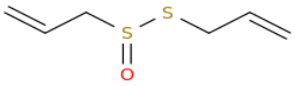
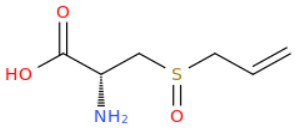
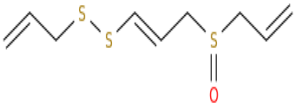
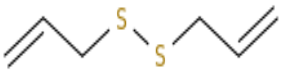
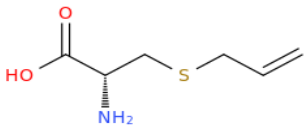
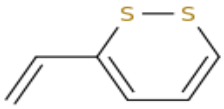
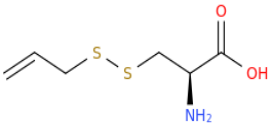
Name	2D Structure	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)
Allicin		3-prop-2-enyl sulfinylsulfanyl prop-1-ene	$C_6H_{10}OS_2$	162.273
Alliin		(2R)-2-amino-3-prop-2-enyl sulfanylpropanoic acid	$C_6H_{11}NO_3S$	177.218
Ajoene		4,5,9-Trithiadodeca-1,6,11-triene 9-oxide	$C_9H_{14}OS_3$	234.39
Diallyldisulfide		3-(prop-2-enyl disulfanyl) prop-1-ene	$C_6H_{10}S_2$	146.266
Diallyltrisulfide		3-(prop-2-enyl trisulfanyl) prop-1-ene	$C_6H_{10}S_3$	178.326
S-allyl-L-cysteine (SAC)		(2R)-2-amino-3-prop-2-enyl sulfanylpropanoic acid	$C_6H_{11}NO_2S$	161.219
3-vinyl-4H-1,2-dithiin		3-ethenyl-3,4-dihydro dithiine	$C_6H_8S_2$	144.254
S-allyl mercaptocysteine (SAMC)		(2R)-3-prop-2-enylsulfanyl-2-(sulfanyl amino) propanoic acid	$C_6H_{11}NO_2S_2$	193.279

TABLE 2. Mechanism/s of actions of organ sulphur compounds of garlic against different diseases.

Diseases	Organosulphur compounds	Mechanism/s of actions	References
Cancer	Allicin	Inhibit cell viability and invasion	[9]
		Reduce cell population in G0/G1 phase	[9]
		Increase 5-FU inducing cytotoxicity	[9]
		Ameliorate the apoptosis	[4]
		Decrease the growth of cells at G2/M phase	[9,11]
		Reduce tumour volume and tumour load	[12]
Inflammation & Arthritis	Allicin	Inhibit of COX-2	[16]
		Suppress HLAB27 protein expression	[19]
	(Z, E)-ajoene	Suppress of LPS-induced production of NO and PGE2.	[12]
		Hinder of the expression of iNOS and COX-2 genes.	
Cardiovascular diseases	Allicin	Regulate the expression of various anti-oxidative enzymes via Nrf2/Keap1 binding	[23,24]
		Inhibit intracellular ROS production	[26,27]
		Increase GPx activities	[27]
		Obstruct Bcl-2/Baxsignaling pathway-dependent apoptosis	[28]
Neuron diseases	Allicin	Act through Akt/eNOS pathway	[30]
		Mediate Nrf2 nuclear translocation	[36]
	SAC	Inhibit calpain via decreasing the level of intracellular Ca2+	[32]
	DADS	Regulate ERK, BDNF and CREB signaling	[35]
	Z-ajoene	Reduce lipid peroxidation	[37]
Microbial diseases	Allicin	Inhibit Bcc by targeting its thiol	[41]
		Show synergistic activity with AgNPs	[43]
		Inhibit urease activity	[44]
Diabetes	Allicin	Reduce autoantibodies, anti-islet cell antibodies	[45]
	SAC	Decrease oxidative stress	[46]
		Activate peroxisome proliferator activated receptor	[47]
High cholesterol	Allicin	Inhibit squalene-monooxygenase, acetyl-CoA synthetase, etc.	[23]
		Suppress apoptosis and oxidative stress pathway	[48]

and anti-islet cell antibodies (ICA) in type 1 diabetes (IDDM). As a result, pancreatic tissues were repaired and the reduced level of insulin was considerably ameliorated in the serum [45].

S-allyl cysteine (SAC) showed protective effects against streptozotocin-induced diabetes in rats by decreasing oxidative stress, normalizing the levels of blood glucose comparable with gliclazide standard [46]. In another study, S-allyl-L-cysteine improved blood glucose levels and normalized the levels of plasma insulin by activating peroxisome proliferator-activated receptor [47].

Cholesterol-lowering activity

According to "low-density lipoprotein (LDL)-receptor hypothesis" cholesterol is responsible for atherosclerosis, and causes the formation of plaques in the arteries. Therefore, it is considered to be a risk factor for atherosclerosis and also for cardiac infarction, angina pectoris or stroke. Researchers have shown that allicin inhibits different enzymes of the cholesterol biosynthesis pathway, namely, squalene-monooxygenase, acetyl-CoA synthetase, etc. to exert a direct impact on cholesterol-metabolism [23]. Allicin blocked oxidized-LDL-induced endothelial cell damage by suppressing apoptosis and oxidative stress pathway and showed a cardiovascular protective effect [48].

Miscellaneous

Allicin was seen to have immuno-modulatory activity. The endogenous immune system, which acts as a defense system, was influenced by allicin. Allicin stimulated the immune processes resulting in transendothelial migration of neutrophils. Finally, the researchers concluded that allicin may be an interesting candidate in the future for allergy or auto-immune disorders [23].

The glutamyl-s-allyl-cysteine peptide of fresh garlic was found to possess radical scavenging and metal-chelating capacities [49]. Garlic saponins can protect dPC12 cells from hypoxia damage [50].

Conclusion

Our literature review has identified many organ sulphur components of garlic and their possible mechanisms for various diseases. Allicin and related organo sulphur compounds from garlic showed potential applications in treating chronic diseases like cancer, inflammation, arthritis, diabetes, cardiovascular disease, neurological diseases, hypercholesterolemia, etc. The information provided in this review will provide strategies for efficient organo sulfur-based treatments of several diseases. Specifically, allicin may be considered as a good chemo preventive agent in the near future, due to its lower cytotoxicity in normal cells compared to tumour cells. However, there is a huge lack report of bioavailability testing, pharmacokinetic study, and toxicology study of these organo sulfur compounds. Another feature that can take a major role in the development of these compounds is their use in clinical trials. Therefore, sufficient studies are needed in this regards. Further research efforts are needed in the future for a clear understanding of the interconnection between these functional components and chronic diseases.

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