Review

Role of garlic in the prevention of ischemia-reperfusion injury

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Garlic in different forms has antioxidant properties. These properties are shown to be due to the existence of compounds such as water soluble organosulfur compounds, S-allylcysteine and lipid soluble compounds like diallyl sulfide. The *in vivo* and *in vitro* ischemia reperfusion studies showed that prophylactic administration of aqueous garlic prior to ischemia reperfusion inhibit lipid peroxidation and prevent depletion in glutathione through its compounds that led to functional recovery. Its ability to inhibit neutrophil migration could suppress fibrosis formation. These preventive effects are seen in models that studied organs such as kidney and liver with functional recovery. Organ system specific activity such as angiotensin converting enzyme-inhibiting activity contributes to a cardioprotective and blood pressure lowering effect. Future studies should focus on post ischemia reperfusion administration of garlic to explore its rescue potential rather than prophylactic impact. Bench research findings should be translated into clinical use through human studies.

Keywords: Garlic / Ischemia / Liver / Oxidative damage / Reperfusion

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1 Introduction

Garlic (*Allium sativum L.)* is an important and widely cultivated plant with both culinary and medicinal uses stemming from its biological activities, which include antibiotic, anticancer, anti-thrombotic, and lipid-lowering cardiovascular effects. Despite such extensive medicinal use of garlic for centuries, there was little scientific support for its therapeutic and pharmacological properties. However, there has been a recent upsurge of research on the therapeutic potential of garlic, aiming to understand its exact mechanism of action in different clinical situations, so that garlic and its products may have more judicious future applications [1].

Garlic is believed to have originated in Central Asia and belongs to the *Alliacae* family. It is used universally as a flavoring agent, traditional medicine, and a functional food to enhance physical and mental health. The beneficial effects of garlic consumption in treating a wide variety of human disease and disorders have been known for centuries; thus, garlic has acquired a special position in the folklore of many cultures as a formidable prophylactic and therapeutic medicinal agent. It is even cited in the Egyptian Codex Ebers, a 3500 year old document, as useful in the treatment of heart disorders, tumors, worms, bites, and other ailments [2].

The major content of garlic (65%) is water, and the bulk of the dry weight is composed of fructose-containing carbohydrates, followed by sulfur compounds, protein, fiber and free amino acids [3]. It also contains high levels of saponins, phosphorus, potassium, sulfur, zinc, moderate levels of selenium and vitamins A and C, and low levels of calcium, magnesium, sodium, iron, manganese, and B-complex vitamins; garlic also has a high phenolic content [4]. Garlic and other members of the *Allium* family are unusual in containing very high levels of organosulfur compounds, and many of the reported beneficial effects of these vegetables have been attributed to these organosulfur compounds.

Since reactive oxygen metabolites (ROM), or free radicals, have been implicated in mediating various pathological processes such as ischemic heart disease [5], peripheral arterial occlusive disease [6], hypertension [7], hyperlipidemia [8] and drug- or chemical-induced toxicities [9 – 12], it

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Abbreviations: AGE; aged garlic extract; **aqGE**, aqueous garlic extract; **CAT**, catalase; **GPx**, glutathione peroxidase; **GSH,** glutathione; **I/R**, ischemia/reperfusion; **MCAO**, middle cerebral artery occlusion; **MDA**, malondialdehyde; **MPO**, myeloperoxidase; **NO,** nitric oxide; **PAF,** platelet activating factor; **ROM**, reactive oxygen metabolites; **SAC**, S-allylcysteine; **SOD,** superoxide dismutase; **TBARS**, thiobarbituric acid reactive substances

is possible to suggest that the beneficial effects of garlic in these conditions may be due to its antioxidant activity [13]. However, the degree of antioxidative efficacy of various garlic preparations was shown to differ according to variations in the chemical structures and standardization procedures [14]. Since garlic is very frequently used in cooking, the question whether cooking destroys the activity of garlic or not was commonly raised. Prasad *et al*. [13] have shown that the heated or unheated garlic extract can both scavenge exogenously generated highly toxic hydroxyl radicals, suggesting that heating does not destroy its activity.

The epidemiological, clinical, and laboratory data have proved that garlic contains many biologically and pharmacologically important compounds, which are beneficial to human health in combating cardiovascular, neoplastic, and several other diseases. Numerous studies are in progress all over the world to develop effective and odorless garlic preparations, as well as to isolate the active principles that may be therapeutically useful [15].

When considering the active compounds of garlic, *in vivo* reactions in the whole body should be taken into account, because antioxidative activity of a compound is caused by its relative electron state. Horrie *et al* [16] demonstrated that aged garlic extract (AGE) prevents the formation of thiobarbituric acid-reactive substances (TBARS) and fluorescent substances during lipid peroxidation of rat liver microsomes. Imai *et al.* [17] compared the antioxidant properties of three garlic preparations and organosulfur compounds in garlic. AGE inhibited the emission of low level chemiluminescence and the early formation of TBARS in a liver microsomal fraction initiated by t-butyl hydroperoxide. However, the water extracts of raw and heat-treated garlic enhanced the emission of low level chemiluminescence. Among a variety of water soluble organosulfur compounds, S-allylcysteine (SAC) and S-allylmercaptocysteine, the major organosulfur compounds found in garlic showed radical scavenging activity in both chemiluminescence and 1,1 diphenyl-2-picrylhdrazyl assays, indicating that these compounds may play an important role in the antioxidative activity of AGE. These components are high in AGE since they form during aging [18, 19].

The oil soluble organosulfur compounds present in garlic, diallyl sulfide, diallyl disulfide, dipropyl sulfide and dipropyl disulfide, and allyl methyl sulfide, have been shown to have free radical scavenging activity and inhibit chemical carcinogenesis [20 – 22]

2 Ischemia/reperfusion (I/R)-induced tissue injury

When a tissue is subjected to ischemia, a sequence of chemical reactions is initiated that may ultimately lead to cellular dysfunction and necrosis (Fig. 1). The organ dysfunction that accompanies I/R injury is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction and necrosis [23]. Although no single process can be identified as the critical event in ischemia-induced tissue injury, most studies indicate that depletion of cellular stores and accumulation of toxic metabolites contribute to cell death. It is necessary to re-establish the blood flow in rescuing ischemic tissues, as this permits both the generation of cell charge and washout of toxic metabolites. However, reperfusion of ischemic tissues also leads to a sequence of events that, paradoxically, injure tissues [24]. Reperfusion leads to reoxygenation and the formation and activation of a variety of humoral mediators of injury and inflammation, including oxygen derived free radicals (*e. g.* superoxide radicals, hydroxyl radicals, hydrogen peroxide), lipid mediators (*e. g.* platelet activating factor (PAF) and leukotriene B_4) as well as polypeptide mediators (*e. g.* C5A).

Superoxide radicals and PAF originate to a large extent from endothelial cells. Endothelial cell dysfunction is thought to be the trigger of reperfusion injury, which leads to a marked reduction in nitric oxide (NO) release [25]. Decreased NO along with chemotactic factors (PAF, LTB4, C5A) promote polymorphonuclear cell recruitment to the reperfusion site and adherence to the dysfunctional endothelium. Furthermore, I/R elicits an acute inflammatory response characterized by activation of polymorponuclear cells, which are known to induce tissue injury through the production and release of ROM and cytotoxic proteins (*e. g.* proteases, myeloperoxidase (MPO), lactoferrin) into extracellular fluid [26]. In other words, ROM play a role in the recruitment of neutrophils into post-ischemic tissue, but activated neutrophils are also a potential source of ROM, enhancing the initial insult by a positive feedback mechanism [23, 24]. Therefore, MPO, which is a neutrophil-specific enzyme for normal neutrophil function [27], is used to define the role of neutrophils in reperfusion tissue injury. Although it is not certain whether neutrophil accumulation and activation are the causes or the result of reperfusion injury, increasing evidence suggests that mesengial cells and neutrophils release chemotactic substances (*e. g.* interleukin 8), which further promote neutrophil migration to the tissue, activate neutrophils, and increase the damage [28, 29].

The result of oxygen radical formation is damage to an array of biomolecules found in tissues, including nucleic acids, membrane lipids, enzymes, and receptors. Membrane-associated PUFAs are readily attached by $^{\bullet}OH$ in a process that leads to peroxidation of lipids, which can disrupt membrane fluidity and cell compartmentation, resulting in cell lysis. Thus, oxygen radical-initiated lipid peroxidation may contribute to the impaired cellular function and necrosis associated with reperfusion of ischemic tissues [30, 31].

Since free radicals play an important role in the I/R injury, it has been suggested that garlic extracts could pro-

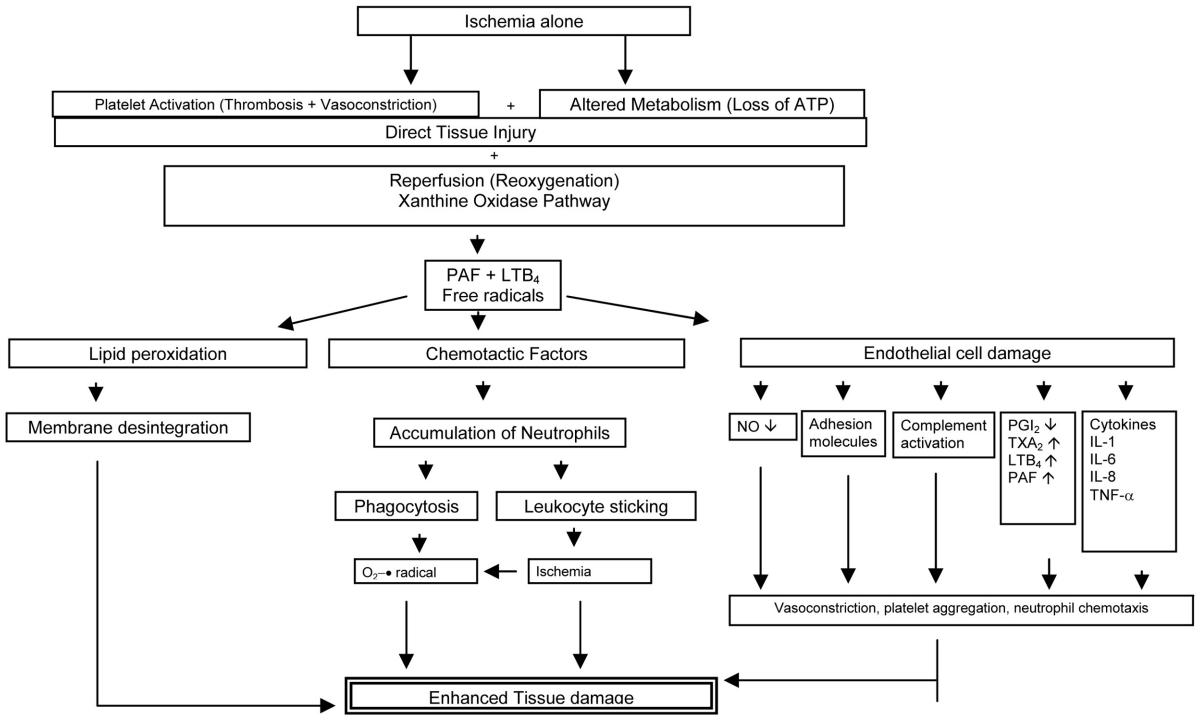

Figure 1. Cascade of events involved in I/R-induced tissue injury.

tect the tissues from the deleterious effects of I/R. Garlic extract and its various components are postulated to have an important cytoprotective role in the setting of I/R injury through their antioxidant and anti-inflammatory properties. This review will focus on the recent research associated with the protective role of garlic in I/R injury of different organs.

2.1 Garlic and renal I/R injury

The temporary discontinuation of renal blood supply is encountered in many clinical situations, such as kidney transplantation [32], partial nephrectomy [33], renal artery angioplasty [34], cardiopulmonary bypass [35], aortic bypass surgery [36], accidental or iatrogenic trauma [37], sepsis [38], hydronephrosis [39], and elective urological operations [28]. All these studies proposed that free radical scavengers could be useful in the clinical settings of I/R damage. Thus, free radical ablation for the treatment of reperfusion injury has found its first clinical application in the prevention of post-ischemic tissue injury following organ transplantation [40, 41].

In the light of this knowledge, we have investigated the possible protective effects of garlic extract against oxidative stress during I/R injury of the kidney, by determining biochemical parameters and histological examination [42]. Wistar albino rats were unilaterally nephrectomized, and subjected to 45 min of renal pedicle occlusion followed by 6 h of reperfusion. Aqueous garlic extract (aqGE, 1 mL/kg, i.p., corresponding to 500 mg/kg) or vehicle was administered twice, 15 min prior to ischemia and immediately before the reperfusion period. The results revealed that I/R induced nephrotoxicity was characterized by a decline in kidney function and an extensive histological damage. Histological analysis revealed severe hemorrhage in the kidney parenchyme prominently in the periphery of the glomeruli, while intertubular interstitium showed severe detachments and there was cellular debris in the proximal tubuli (Fig. 2a)). In most areas, the glomeruli had lost their normal morphology. However, in the aqGE-treated I/R group, detachment and hemorrhage of the parenchyme were no longer observed and the glomerular morphology retained its integrity with minimal cellular debris in the tubuli (Fig. 2b)). I/R-induced increase in malondialdehyde (MDA), an end product of lipid peroxidation, in the rat renal tissue was reversed by aqGE (Fig. 3). Garlic, that contains thioallyl compounds, prevented the depletion in the intracellular antioxidant glutathione (GSH) and, in turn, maintaining the intracellular GSH level most likely reduced the injury from oxidative damage. In addition, I/R-induced increase in renal tissue collagen content, indicating renal fibrosis, was reduced by aqGE, suggesting its preventive effect on inflammation-induced production of extracellular matrix components. Moreover, aqGE decreased the infiltration of neutrophils, major source of free-radical-induced tissue injury, as assessed by a reduction in MPO activity. As a

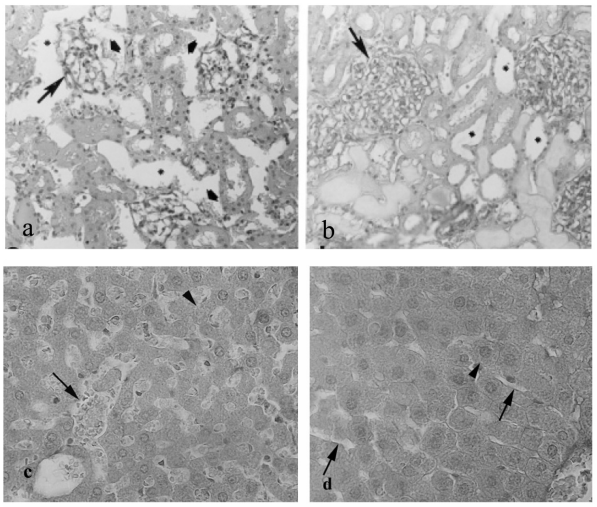

Figure 2. KIDNEY: a) *I/R group,* severe hemorrhage prominent in the glomeruli (arrowheads), degenerated structure of a glomerulus (arrow) and widespread cellular debris in the tubuli (*), b) *I/R + aqGE group,* regenerated tubuli (*) and a glomerulus with urinary space (arrow) with decreased interstitial edema. H&E staining, ×200 magnification [42]. LIVER: c) *I/R group:* Severe sinusoidal congestion (→) and hemorrhage, dilated central vein and degenerated hepatocytes (▲) showing perinuclear vacuolization; d) *I/R + aqGE group:* Moderate sinusoidal dilatation (→) and hemorrhage in localized areas, with the usual appearance of central vein and hepatocytes (▲) in most areas. H&E staining, scale bar: 10 μm [46].

result of garlic's antioxidant and anti-inflammatory features, kidney functions were nearly restored by aqGE treatment (Table 1).

Table 1. aqGE improves renal function in I/R injury [42]

	Control	aqGE	I/R	I/R + aqGE
Creatinine	0.53 ± 0.04	0.62 ± 0.03	1.161 ± 0.08[a)]	0.73 ± 0.10[b)]
BUN	38.0 ± 0.24	35.8 ± 0.26	110.7 ± 4.5[a)]	64.8 ± 7.8[c), d)]

a) $p < 0.001$: compared to control group
b) $p < 0.01$: compared to saline-treated I/R group
c) $p < 0.01$: compared to control group
d) $p < 0.001$: compared to saline-treated I/R group
BUN, blood urea nitrogen

2.2 Garlic and hepatic I/R injury

The liver is highly sensitive to I/R injury, which occurs clinically during circulatory shock [43], disseminated intravascular coagulation [44] and surgery involving this organ, including liver transplantation [45]. There has been a complete lack of literature on the role of garlic in the setting of I/R injury to the liver.

We have recently examined the effects of aqGE on the hepatic I/R injury in rats [46]. Elevated hepatic enzymes seen in I/R groups were returned to normal by the intraperitoneal administration of aqGE (1 ml/kg, i.p., corresponding to 500 mg/kg) prior to ischemia and reperfusion (Table 2). In accordance with these, lipid peroxidation characterized by the increase in MDA was significantly reduced by aqGE, while the reduction in the endogeneous antioxidant GSH was prevented (Fig. 4). Light microscopic examination of the hepatic tissue with I/R injury revealed severe sinusoidal congestion, hemorrhage, edema and degeneration of hepatocytes, where as aqGE- treated group only had moderate sinusoidal dilatation and hemorrhage with well preserved liver damage (Fig. 2c and d). In this single study,

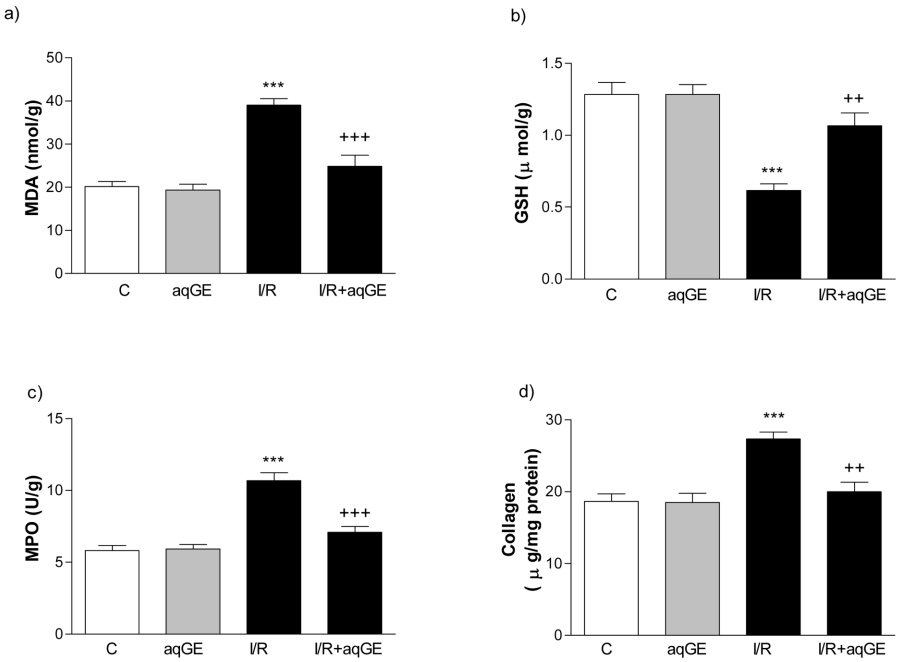

Figure 3. The effect of I/R and aqGE treatment on the renal tissue a) MDA level, b) GSH level c) MPO activity, and d) collagen content.
***$p < 0.001$; compared to control group. +++$p < 0.001$; compared to saline-treated I/R group [42].

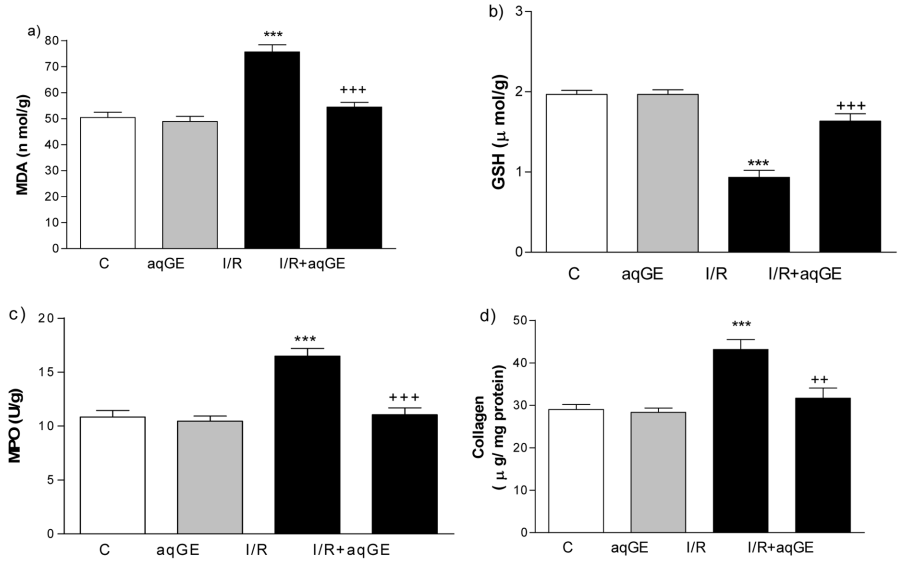

Figure 4. The effect of I/R and aqGE treatment on the liver tissue a) MDA level, b) GSH level c) MPO activity, and d) collagen content. *** $p < 0.001$; compared to control group. +++$p < 0.001$; ++$p < 0.01$; compared to saline-treated I/R group [46].

Table 2. The effect of aqGE on plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in control, aqGE-treated, I/R and I/R + aqGE-treated groups [46]

	AST (U/L)	ALT (U/L)
control group, (n=8)	242 ± 7.5	108 ± 7.5
aqGE group, (n=8)	228 ± 9.2	106 ± 7.4
I/R group, (n=8)	2116 ± 80.3[a)]	1995 ± 152.7[a)]
I/R + aqGE group, (n=8)	578 ± 30.6[a), b)]	347 ± 28.5[b)]

a) $p < 0.001$: Compared with control group.
b) $p < 0.001$: Compared with I/R group.

we have demonstrated that the functional, biochemical and structural changes seen in this hepatic I/R rat model due to various factors, especially formation of free radicals, lipid peroxidation and migration of neutrophils, were reversed by preemptive administration of aqGE via its antioxidant and anti-inflammatory properties [46].

2.3 Garlic and cardiac I/R injury

The susceptibility to ventricular arrhythmias under the conditions of cardiac ischemia and reperfusion was investigated by Rietz *et al.* [47]. They studied the Langendorff heart preparation of rats fed for eight weeks a standard chow enriched with 2% of pulverized wild garlic leaves and showed that the incidence of ventricular fibrillation during 20 min occlusion of the descending branch of the left coronary artery was significantly reduced in the garlic-fed group as compared to untreated controls (20 *vs* 88%). A similar reduction was also found in the size of the ischemic zone (33.6 *vs* 40.9% of heart weight). In the reperfusion experiments (5 min after 10 min ischemia), ventricular tachycardia, which was 100% in untreated controls, occurred in 70% of the garlic-fed group. Similarly, ventricular fibrillation was also found to be decreased. The time until occurrence of extrasystoles, venricular tachycardia or ventricular fibrillation was prolonged. They also showed that prostacyclin production was slightly increased in hearts of the garlic-fed group. However, inhibition of cyclooxygenase by acetylsalicylic acid could not completely prevent the cardioprotective effects of garlic, suggesting that the prostaglandin system does not play a decisive role in the cardioprotective action of wild garlic. Furthermore, wild garlic was found to inhibit angiotensin converting enzyme activity both *in vitro* and *in vivo*. Thus, angiotensin converting enzyme-inhibiting action of garlic appears to contribute to its cardioprotective and blood pressure lowering effects.

Ischemic preconditioning is a unique myocardial adaptive mechanism that develops after brief periods of I/R prior to longer duration of myocardial ischemia and reperfusion [48]. Allitridum, an extract of garlic is reported to promote ischemic preconditioning in the rabbit heart subjected to 30 min of regional myocardial ischemia and 120 min of reperfusion [49]. This study clearly showed that "pharmacological preconditioning" in hearts with a 5 min allitridum infusion 10 min before the prolonged regional ischemia resulted in significantly smaller infarcts (7 ± 6% of risk area) than in control hearts (25 ± 7%, $p < 0.05$).

Chronic garlic administration was also shown to prevent oxidative stress and associated ultrastructural changes induced by myocardial I/R injury [50]. In the study of Banerjee *et al.* [51] raw garlic homogenate (125, 250 or 500 mg/kg once daily for 30 days) was administered orally to Wistar albino rats. Thereafter, hearts were isolated and subjected to I/R injury with 9 min of global ischemia, followed by 12 min of reperfusion, resulting in significant myocyte injury and a rise in myocardial TBARS along with the reduction in myocardial superoxide dismutase (SOD), catalase (CAT), GSH and glutathione peroxidase (GPx). On the

other hand, depletion of myocardial endogenous antioxidants and the rise in TBARS were significantly less in the garlic-treated rat hearts. Oxidative stress-induced cellular damage as indicated by ultrastructural changes, like disruption of myofilament, Z-band architecture along with mitochondrial changes were significantly less in the garlic-treated groups.

Apart from studies using aqueous garlic preparations, some studies have evaluated the role of garlic oil in the treatment of several disease states. Garlic oil treatment given to the patients with coronary heart disease demonstrated that garlic significantly ($p < 0.01$) reduced heart rate at peak exercise and also significantly reduced the work load upon the heart, resulting in a better exercise tolerance ($p < 0.05$), as compared to their initial tests [52]. Although a larger study is required to test their validity, similar results came from the study of Zhang *et al.,* who demonstrated that most people taking garlic supplements lacked overt risk of coronary heart disease [53].

Another study that investigated the antioxidant role of garlic oil in rats has demonstrated a marked reversal of the metabolic changes related to myocardial infarction induced by isoproterenol that was shown to act by modulating lipid peroxidation and enhancing antioxidant and detoxifying enzyme systems [54].

2.4 Garlic and cerebral I/R injury

Numagami *et al.* [55] studied the effects of an AGE and its thioallyl components on rat brain ischemia using a middle cerebral artery occlusion model and a transient global ischemia model. In focal ischemia, an AGE, SAC, allyl sulfide or allyl disulfide was administered 30 min prior to ischemic insult. Three days after ischemic insult, water contents of both ischemic and contralateral hemispheres were measured to assess the degree of ischemic damage. The water content in the ischemic control without treatment (81.50 ± 0.07%) was significantly reduced with the administration of 300 mg/kg of SAC (80.66 ± 0.11%; $p < 0.001$). The histological observation demonstrated that the administration of SAC reduced the infarct volume, however neither allyl sulfide nor allyl disulfide was effective. They also studied the global ischemia, where the production of ROS showed two peaks; first at 5 min and second at 20 min after reperfusion. Neither SAC nor 7-nitro indazole, a NO synthase inhibitor, attenuated the amount of ROS produced at the first peak, but both could reduce the amount of ROS at the second peak. A possible involvement of peroxynitrite, which may be formed from superoxide and NO, is known to be highly toxic in I/R injury of the brain, was suggested [55].

Similarly Saalem *et al*. [56] studied the effects of aqGE on neurobehavioral activities, MDA, GSH levels, GPx, glutathione reductase, glutathione S-transferase, SOD, CAT, and sodium-potassium ATPase (Na^+, K^+-ATPase) activities, and glutamate and aspartate content in a middle cerebral artery occlusion (MCAO) model of acute cerebral ischemia in rats. MCAO caused significant depletion in GSH and its dependent enzymes (GPx, glutathione reductase, and glutathione S-transferase) and significant elevation of MDA, glutamate, and aspartate. The activities of Na^+, K^+-ATPase, SOD, and CAT were decreased significantly by MCAO where neurobehavioral activities (grip strength, spontaneous motor activity, and motor coordination) were also decreased. On the other hand, all of the alterations induced by MCAO and reperfusion were significantly attenuated by pretreatment with aqGE (500 mg/mL/kg of body weight, i.p.) 30 min before the induction of MCAO and these results correlated well with the histopathological findings showing decreased neuronal cell death. These findings suggest that aqGE effectively modulates neurobehavioral and neurochemical changes in focal ischemia, most probably by virtue of its antioxidant properties.

In the setting of cerebral I/R, in addition to free radical generation, local production of prostaglandin E_2 and leukotrienes may induce vasogenic edema that could lead to damage, while aqGE may ameliorate the injury by reducing these local mediators [57]. In a model of global cerebral ischemia induced by occlusion of right and left common carotid arteries, garlic oil given prior to ischemia markedly reduced the cerebral infarct size and attenuated some functional impairments, such as defects in short-term memory and motor coordination. This effect was accompanied by a significant decrease in mitochondrial oxidative stress, as assessed by a reduced level of TBARS [58].

2.5 Garlic and pulmonary I/R injury

As it is true for the other organs, reducing I/R-associated pulmonary injury is of critical importance to allow for a successful lung transplantation after several hours of ischemia. There is only one study reported by Batirel *et al.* [59] that explored the anti-oxidant role of garlic in pulmonary I/R injury. I/R was established by clamping one pulmonary artery for 1 h, followed by reperfusion for 2 h while clamping the opposite pulmonary artery, to reflect solely the function of the single lung. In the rats that received allicin, a sulfur-containing compound extracted from garlic, a decrease in pulmonary vascular resistance and a sudden increase in pulmonary blood flow were observed.

3 Conclusion

Garlic, in the fresh or aged forms, has been used as a folk remedy for various disorders for thousands of years. Animal I/R studies suggest that garlic with its antioxidant and anti-inflammatory properties, is a potential therapeutic agent to protect the function and structure of various organs against the injury from oxidative damage and neutrophil

infiltration. Further basic science and clinical studies are needed to translate this potential to clinical use.

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