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Benefits versus toxicities of garlic (*Allium sativum*) in dogs: Review

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Abstract. Garlic (*Allium sativum*) is a plant with therapeutic/toxic properties in dogs, depending on the variety, place of origin, its preparation method or extraction method of its compounds and the dose administered. There are reports in preclinical and clinical investigations that active organosulfur compounds (OSCs) of garlic potentially exerts a series of beneficial activities in dogs. such as being an endoparasiticide, antithrombotic, hypotensive, diuretic and natriuretic, antimicrobial, cell antiproliferative, antioxidant, activator of the Nrf2 transition factor, antiarrhythmic, immuno modulator, hypoglycemic, hypolipidemic and ectoparasiticide. While they also potentially exert toxic effects on gastrointestinal system and erythrocyte cells of dogs. However, there is still no review on its possible beneficial and toxic effects. or hypotheses of its underlying mechanisms in dogs. The aim of this paper was to summarize the preclinical and clinical investigations on the effects of garlic and its OSCs in dogs, as well as the findings on their possible proven or presumed underlying mechanisms. We conclude that further in vivo and toxicity studies are needed to prepare specific herbal formulations for dogs with relatively low concentrations of garlic phytochemicals. We also emphasize that, regardless of the preparation method, dog owners should be advised that it is not safe to provide or add garlic to their dogs' food.

Keywords: functional food, organosulfur compounds, immunomodulators, medicinal plants

Benefícios versus toxicidades do alho (*Allium sativum*) em cães: Revisão

Resumo. O alho (*Allium sativum*) é uma planta com propriedades terapêuticas/tóxicas em cães, dependendo da variedade, do local de origem, da forma de preparo ou do método de extração utilizado e da dose administrada. Há relatos em investigações pré-clínicas e clínicas de que o alho ou seus compostos organossulfurados ativos (OSCs) exercem potencialmente uma série de atividades benéficas em cães: endoparasiticida, antitrombótico, hipotensivo, diurético e natriurético, antimicrobiano, antiproliferativo celular, antioxidante, ativador do fator de transição Nrf2, antiarrítmico, imunomodulador, hipoglicêmico, hipolipidêmico e ectoparasiticida. Bem como toxicidade no sistema gastrointestinal e eritrócitos com ação hemolítica em cães. No entanto, ainda não há revisão sobre seus possíveis efeitos benéficos e tóxicos e hipóteses de seus mecanismos subjacentes em cães. O objetivo deste artigo foi resumir as investigações pré-clínicas e clínicas sobre os efeitos do alho e seus OSCs em cães, bem como as descobertas sobre os possíveis mecanismos subjacentes comprovados ou presumidos. Concluímos que mais estudos in vivo e de toxicidade são necessários para preparar formulações herbais específicas para cães com doses relativamente baixas de fitoconstituintes de alho. Também enfatizamos que, independentemente do método de preparação, os donos de cães devem ser avisados de que não é seguro fornecer ou adicionar alho à comida de seus cães.

Keywords: functional food, organosulfur compounds, immunomodulators, medicinal plants

Introduction

Since ancient times, most of the world has been practicing traditional and popular medicine using medicinal plants or herbs to preserve health and well-being ([Afsheen et al., 2018](#); [Almeida, 2011](#); [Batista](#)

[et al., 2017](#); [Chavan et al., 2016](#); [Haraguchi & Carvalho, 2010](#)). One of its branches developed ethnoveterinary medicine (EVM), which refers to the knowledge and therapeutic practices applied to animals, providing a lower-cost alternative treatment ([Lans, 2019](#); [Orengo, 2016](#); [Silva & Fernandes Júnior, 2010](#)).

Some food constituents are considered particularly functional because they selectively promote the alteration of specific physiological processes, benefiting health or preventing diseases ([Chang et al., 2004](#)). Plants or herbs generally have multiple uses, in addition to being used in cooking, they contain a wide diversity of phytochemicals with medicinal properties, which can potentially promote the homeostasis of beings ([Ezeorba et al., 2022](#); [Salgado et al., 2011](#)).

For centuries, the bulbils of *Allium sativum* are used not only as a condiment and flavoring culinary, but also as a health promoter and disease preventer ([Amano et al., 2015](#); [Chang et al., 2004](#); [Chen et al., 2009](#); [Hu et al., 2002](#); [Mosallanejad et al., 2013](#); [Phanasasophon et al., 1995](#)). There is evidence in the Traditional Chinese Medicine that garlic has been used to preserve human health for over 3,000 years ([Ezeorba et al., 2022](#)). Garlic (*Allium sativum* Linn.) now belongs to the family Amaryllidaceae of the subfamily Alliioideae ([Salgado et al., 2011](#)). The term sativum means “cultivated” ([Orengo, 2016](#)).

The chemical composition of garlic is diverse: water (65%), fiber, adenosine, pectin, lectins, fructans, nicotinic acid, flavonoids, fatty acids, phospholipids, prostaglandins, enzymes, vitamins, minerals such as selenium, amino acids, and sulfur compounds ([Ezeorba et al., 2022](#); [Ivanova et al., 2009](#); [Lans, 2019](#); [Orengo, 2016](#)). The metabolomic profile of *Allium sativum* identified the presence of the flavonoids myricetin and apigenin ([Afsheen et al., 2018](#)). Freshly harvested or intact garlic bulbs contain a colorless, odorless solid, its most abundant compound (0.24% by weight): the alliin, S-allyl-cysteine sulfoxide (SACS) ([Ezeorba et al., 2022](#); [Orengo, 2016](#); [Pantoja et al., 1991](#)). The traumatic destruction by cut or crushing of its plant tissues, allows the release of alliinase enzyme what converts alliin into the unstable allicin (diallyl thiosulfinate) ([Lans, 2019](#); [O’Gara et al., 2000](#); [Pantoja et al., 1991](#)). Allicin is an odoriferous, lipophilic substance, considered the main active agent in freshly crushed garlic homogenates, and can be isolated by extraction with ethanol at room temperature ([Chavan et al., 2016](#); [Mosallanejad et al., 2013](#); [Orengo, 2016](#); [Pantoja et al., 1991](#)). Phyto therapeutics have almost no side effects, they have bigger availability, lower cost than synthetic drugs ([Afsheen et al., 2018](#); [Chang et al., 2004](#); [Chavan et al., 2016](#)) and a possible alternative to the indiscriminate use of antibiotics ([Lans, 2019](#)).

The development of new Phyto therapeutics depends on the analysis of the metabolomic profile of the medicinal plant together with the study of evidence on the effect of its active secondary metabolites ([Afsheen et al., 2018](#)). In research to confirm the properties and mechanisms of action of functional foods for pets in MEV, garlic is one of the medicinal plants with potential to be incorporated as a dietary supplement ([Chang et al., 2004](#); [Lans, 2019](#); [Orengo, 2016](#); [Yamato et al., 2018](#)).

The aim of this work was to summarize preclinical and clinical investigations on the effects of garlic and its OSCs in dogs, as well as findings on possible proven or presumed underlying mechanisms.

Methodology

A thorough literature search was conducted using multiple databases including PubMed, Google Scholar and Science Direct to search for literature on the action of garlic and its OSCs in dogs, including articles up to November 2023 in this review. Studies on the effects of garlic and its OSCs were selected if they covered the following information: (i) in vivo studies, (ii) in vitro studies, (iii) clinical trials of garlic in canine conditions (iv) studies that included garlic extracts or its isolated OSCs and (v) studies that incorporated the possible mechanisms of action of garlic and its OSCs.

The variety of *Allium sativum* used affects its effect

The herbaceous garlic plant began to be cultivated more than 5,000 years ago in cold climates in Central Asia ([Resende et al., 2015](#)), but is now planted in all parts and in different climates of the world ([Pantoja et al., 1991](#)). This has led to the various varieties of this vegetable, with bulbs containing 5 to 56 cloves, which are classified according to their size: large (4-6 g), medium (3 g) and small (2 g) ([Resende et al., 2015](#)).

Several factors interfere both in the characteristics of the garlic bulb and in the proportions of its phytoconstituents, which will determine its therapeutic/toxic properties ([Ezeorba et al., 2022](#); [Phanasasophon et al., 1995](#)). Some of these factors are: geographic location with its different photoperiods and environmental challenges, which may or may not direct gene expression in the production of phytochemicals ([Ezeorba et al., 2022](#)) or phytotoxins ([Nagy et al., 2023](#)).

A study in Thailand replicated the experiment by [Pantoja et al. \(1991\)](#), using 5 dogs for each dose administered, although the 4 doses of dried garlic powder (100, 300, 600 and 1200 mg/kg) were significantly higher, they did not obtain the same hypotensive effect. This study claims that this variable action may be due to the different origin of the garlic plant varieties with different proportions of phytochemicals and/or the different lineage of dogs used in the experiment ([Phanasasophon et al., 1995](#)).

It was observed in a study that garlic from different geographical locations produced different proportions of alliin, methiin and flavonol. Other researchers have isolated different concentrations of alliin between garlic germ sperms from Egypt and China, significantly reducing its concentration when the Egyptian germ sperm was grown in China ([Ezeorba et al., 2022](#)).

The pharmacokinetics and metabolism of garlic OSCs in dogs interfere with their effect

Aged garlic extract (AGE) is prepared by immersing fresh garlic bulbs in aqueous ethanol for an extended period of up to 20 months at room temperature ([Amano et al., 2016](#); [Takahashi et al., 2023](#)). AGE OSCs are bioactive amino acids: S-allyl-cysteine (SAC), trans-S-1-propenyl-cysteine (S1PC), S-allyl-mercapto-cysteine ([Takahashi et al., 2023](#)) and S-methyl-cysteine (SMC) ([Amano et al., 2016](#)). It is hypothesized that the compounds S1PC and SAC, which have a similar chemical structure and the same proportion contained in AGE, are formed through the enzymatic transformation of their γ -glutamylated precursors ([Amano et al., 2016](#)). The biological and pharmacological properties of AGEs were analyzed by studying the pharmacokinetics of their OSCs: SAC, S1PC and SMC in dogs and their in vitro metabolism in the S9 fractions of canine kidney and liver ([Amano et al., 2015, 2016](#)).

With oral administration of 2 mg/kg of SAC or S1PC, bioavailability of 92% and 100% was obtained, respectively. There was very low urinary excretion of both the unchanged forms SAC/S1PC and its metabolites: N-acetyl-(Nac-)SAC/Nac-S1PC, SAC sulfoxide (SACS) and Nac-SACS/Nac-S1PCS sulfoxide. The liver, according to in vitro studies, exerts a relative activity in N-acetylating SAC/S1PC and alliin (SACS) ([Amano et al., 2015, 2016](#)). A new metabolite of SAC, L- γ -glutamyl-SAC (GSAC) was also detected in plasma ([Amano et al., 2015](#)). The plasma concentration of Nac-SAC/Nac-S1PC was 1/2 to 1/3 of that of SAC/S1PC, being its main metabolite. The extremely low values of plasma clearance (CL) and renal clearance (CLr) of SAC/S1PC indicated its extensive renal reabsorption. In vitro metabolism suggests that there is a high renal activity in deacetylating Nac-SAC/Nac-S1PC, converting it to SAC/S1PC. This high reabsorption associated with the low urinary excretion of its N-acetylated forms may explain the long half-life ($t_{1/2}$) of 12h for SAC and 5.3-5.6h for S1PC. This study suggests that the delay in the elimination of SAC/S1PC by the kidney favors its repeated recirculation systemically to finally be excreted in the bile ([Amano et al., 2015, 2016](#)) In the S-oxidation metabolism of SAC in vivo, there was the recovery of alliin (SACS) present in raw garlic.

The compound SMC and its metabolites: Nac-SMC and Nac-SMCS are produced endogenously in dogs, as they were present in basal concentrations in plasma and urine. With SMC administration, a bioavailability of 95.5% was found. The low CL and CLr values of SMC found indicated its extensive renal reabsorption, explaining its low urinary excretion as well as its long $t_{1/2}$ of 8.0–8.9 h. In the liver, in vitro studies showed no N-acetylation activity of SMC and there is a high activity to deacetylate Nac-SMC in both the liver and kidney, which would explain the basal plasma concentration of SMC ([Amano et al., 2016](#)).

Beneficial activities of garlic or its OSCs in canines

Garlic extracts, because they contain bioactive compounds, have the ability to act on pathogens or systemic imbalances and directly or indirectly activate the body's defense system ([Vidal & Pereira, 2012](#)).

The active phytochemicals/phytotoxins in garlic can be extracted or eliminated in the various forms of its preparation or in its variables such as temperature and solvent of the extraction method, affecting the quality, the proportion of phytochemicals and the yield of the extract (Ezeorba et al., 2022; Pantoja et al., 2000). Some of the work reported here was carried out in an attempt to draw a parallel between the action of garlic between dogs and humans, but the results obtained indicate that the metabolism and enzymatic system of rats represent a better model for the human species than those of dogs (Amano et al., 2015, 2016; Chang et al., 2004)

Activity on endoparasites

Research points to some of the OSCs in *Allium sativum* extracts that have antiparasitic effects: ajoene, allicin, allyl-methyl thiosulfanate and ethyl-allyl thiosulfanate, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) (Orengo, 2016).

Treatment with 7-10g/day (46g total) of raw garlic mixed with the food of a young 10 kg dog parasitized by *Ancylostoma caninum* for 5 days had no effect on reducing the percentage of egg shedding in feces. However, during the treatment period, there was a significant reduction in the survival of developing larvae; some dead eggs at various stages of development were also observed in the fecal culture, which returned to the initial level 2 days after the end of treatment. This study indicates that garlic has the potential to inhibit the hatching and development of larvae of *Ancylostoma caninum* (Bastidas, 1969). Although the daily dose used is relatively small, approximately that of a bulb of raw garlic (1 g/kg), it was not carried out with an appropriate sample nor was it reported whether there were clinical changes in the dog.

In an *in vitro* study, the same amount of dried garlic powder (sourced from Nairobi, Kenya) was used to prepare two extracts: cooked aqueous and 99.5% ethanolic to test their anthelmintic activities on feces of puppies naturally infected with helminths. The lyophilized aqueous extract showed moderate effects on the hatching/development of eggs and survival of larvae of *Ancylostoma caninum* and *Toxacara canis*. While the ethanolic extract paste at a concentration of 5,000-10,000 µg/ml inhibited 100% of egg hatching and at 625 µg/ml or more caused 100% mortality of *Ancylostoma caninum* larvae. However, the same effect on the development of *Toxacara canis* eggs was not obtained at the same concentrations (Orengo, 2016). As there are losses or selection of garlic phytochemicals depending on the preparation method or extraction technique used, despite appearing to have a low spectrum, further studies would be needed to determine the effect of garlic on canine helminths.

Antithrombotic activity

Crude garlic extract contains some platelet-inhibiting compounds such as ajoene, allicin and methylallyl trisulfide (MATs), but these compounds evaporate when heated or dried in vacuo, as they are volatile (Chang et al., 2004). Ajoene, which originates from the decomposition of allicin, inhibits platelet aggregation by capturing sulphhydryl groups from their membranes (Pantoja et al., 1991). In low-polarity solvents, the allicin is transformed mainly in vinyl dithiine, one family of volatile OSCs which have antithrombotic properties (Pantoja et al., 1991, 2000).

A canine model of partial coronary artery stenosis with intimal damage was developed and applied to 8 dogs. After observing platelet thrombus formation for 30 minutes, 10% cooked ethanolic aqueous garlic extract (equivalent to 5 garlic bulbs) was administered intravenously, inhibiting thrombus formation in 7 dogs. After 20 minutes, a slow infusion of epinephrine was performed, renewing thrombus formation in 5 of these dogs (DeBoer & Folts, 1989). Although it was not reported whether there were any clinical changes or whether the dogs were sacrificed after the experiment, the administration of cooked garlic extract equivalent to 5 garlic cloves showed antithrombotic activity in most of the dogs used.

The compound 2-propenyl sodium thiosulfate (2PTS), which is heat stable because it is non-volatile, was isolated and identified in cooked garlic extract (Yamato et al., 2003). However, cooked garlic extract had shown a reduced effect on inhibiting platelet aggregation compared to its raw extract (Chang et al., 2004). In the incubation of canine platelet-rich plasma (PRP) with dose-dependent 2PTS, after induction with adenosine 5'-diphosphate (ADP), the lowest platelet aggregation rate was obtained at a dose of 0.01

mM, returning to the level of the control group at higher doses of 0.1-1 mM. Therefore, 2PTS may potentially be an antithrombotic. The antithrombotic effect of 2PTS at relatively low doses with a tendency for loss of effect at higher doses would be an advantage for adding it as a supplement ([Chang et al., 2004](#)).

Platelets express the enzyme cyclooxygenase-1 (COX-1), which by its action generates the endogenous aggregation factor, thromboxane A₂. It is hypothesized that the platelet aggregation inhibitory property of garlic occurs secondary to the inhibition of COX in the biosynthesis of prostaglandins ([Chang et al., 2004](#)). There are also reports that the elevation of the concentration of reduced glutathione (GSH) cellular decreases the production of prostaglandins and its depletion accelerates their production. In the incubation of canine PRP with 2PTS, dose-dependent inhibition of platelet COX activity and a significant decrease in GSH concentration occurred at doses of 0.1mM and 1mM, returning COX activity to the level of the control group at doses above 1mM. In the incubation Cell-free COX-1 with dose-dependent GSH, there was a decrease in COX activity to 27% of the initial at a concentration of 0.1 mM GSH. Therefore, it was deduced that 2PTS may have a modulating effect on platelet aggregation in dogs by inhibiting COX activity, influencing GSH concentration. Intracellular GSH appears to be a modulator that can reversibly inhibit COX activity, suggesting that the decrease in GSH concentration induced by 2PTS could affect COX activity. This study assumes that the action of 2PTS at relatively low doses is safe and can be used as a functional compound ([Chang et al., 2005](#)).

Diuretic and natriuretic activity

When administering dry garlic powder capsules (2.5 to 15 mg/kg) dose-dependently via the intragastric route to anesthetized dogs, there was a significant induction of diuretic (1.9 times the basal value) and natriuretic responses, which returned to the basal level after 2 hours, and were not observed in the control group ([Pantoja et al., 1991](#)).

In the four-step processing of the crude homogenate of pre-cooked garlic, a highly purified fraction with diuretic and natriuretic activity was obtained, and the fraction with hypotensive activity was separated chromatographically from the compound. The mean urinary flow and natriuretic activity were determined by furosemide (0.1 mg/kg), defining a diuretic unit (DU) and a natriuretic unit (NU) equivalent to 2.6 ml/min and 0.87 mEq/min, respectively. Aliquots of the purified fraction (6 mg/kg) were infused intravenously into 8 anesthetized mongrel dogs, inducing a significant biphasic diuretic-natriuretic response equivalent to 6.2 DU and 3.2 NU, which reached a maximum after 3 hours. These results were higher than those found for the crude extracts and the less purified fractions. There was also a loss of chloride ion in the same proportion. No changes in systemic blood pressure (SBP) or electrocardiogram (ECG) were observed. Sodium reabsorption is affected by the levels of the sodium-potassium pump enzyme (Na⁺/K⁺-ATPase) between the different tubular segments of the nephron. Incubation of 25 mL of the rat renal medulla cell membrane fraction with dose-dependent aliquots of this same highly purified fraction in vitro for 15 min at 37°C induced inhibitory effects on renal Na⁺/K⁺-ATPase, which reached 70% inhibition at the highest aliquot concentration (200 mg/ml) compared to the control. Given these results, this study suggests that the diuretic and natriuretic responses induced by the purified fraction occurred through inhibition of Na⁺/K⁺-ATPase in the renal tubular segments ([Pantoja et al., 2000](#)).

Hypotensive activity

In research on the hypotensive effect of garlic, dried garlic powder (ASP) or its aqueous extracts have been mainly used, as they contain allicin and/or DADS ([Phasanasophon et al., 1995](#)). When ASP capsules (15 mg/kg) were administered intragastrically to anesthetized dogs, there was a significant decrease in SBP sustained for up to 3 hours, which was not observed in the control group ([Pantoja et al., 1991](#)). This decrease in SBP may have occurred due to the natriuretic and diuretic effects found in this experiment. Slow intravenous infusion of a dose-dependent pre-cooked garlic dialysate (16.8-67.2 mg/kg) in 10 anesthetized dogs caused rapid hypotension in diastolic blood pressure (DBP) and a sinus bradycardic rhythm in heart rate (HR). At a dose of 67.2 mg/kg of dialysate, there was a significant reduction in DBP (38%) and HR (17%), which returned to baseline levels within 40 s. In the decrease in DBP (from 112.5 ± 3.67 to 70 ± 3.16 mmHg) and HR (from 198 ± 4-9.81 to 164 ± 4-16.59 beats/min),

the correlation coefficients presented were 0.93 and 0.79, respectively. The effect of hypotension associated with bradycardia induced by garlic dialysate, being similar to the action of acetylcholine, was not blocked by the administration of atropine (data not shown). This study suggests that the inhibitory activity on cardiovascular function of garlic dialysate is related to a beta-blocking action ([Martín et al., 1992](#)).

A study in Thailand, using 5 dogs under anesthesia for each dose administered intragastrically of aqueous garlic extract (25 and 50 ml/kg) and oily garlic extract (16, 32 and 48 mg/kg), induced a slight, non-significant reduction in SBP in both the treated and control groups, which was attributed to the effect of the anesthetic. This study claims that the origin of the garlic variety with different proportions of its phytochemicals, the breed of the dogs and/or the dogs being normotensive could be responsible for the unfavorable effect in relation to other studies. When DADS (34 mg/kg/Aldrich) was administered intragastrically to 5 dogs, there was a slight, non-significant reduction in SBP in relation to the control group, which was considered to be the effect of the anesthetic. This study suggests that the hypotensive effect is caused by other phytochemicals in garlic or that the isolated use of DADS has no hypotensive effect in dogs ([Phasanasophon et al., 1995](#)).

Antiarrhythmic activity

In the infusion of ouabain in 9 anesthetized dogs, there was iatrogenic induction of premature ventricular contraction (PVC) type arrhythmia in 5 dogs and ventricular tachycardia (VT) in the other dogs. Both dogs reestablished sinus rhythm after infusion of a pre-cooked garlic dialysate extract in low doses (52.00 ± 28.20 mg/kg) in the PVC and high doses in the VT (125.00 ± 20.30 mg/kg), maintained for approximately 20 and 3 minutes, respectively. This study demonstrated antiarrhythmic activity in relatively low doses of pre-cooked garlic dialysate in dogs ([Martín et al., 1992](#)).

Immune modulation activity

Neutrophils in the defense process, when activated, begin to consume oxygen (O_2) to form superoxide (O_2^-) by the action of reduced nicotinamide adenine dinucleotide phosphate oxidase (NADPH). Phorbol 13-acetate-12-myristate (PMA) stimulates the activation of cellular NADPH oxidase, producing O_2^- and hydrogen peroxide (H_2O_2). In the incubation of canine peripheral polymorphonuclear leukocytes (PMNs) with dose-dependent 2PTS, when stimulated with PMA, it promoted a significant increase in O_2^- generation and a significant reduction in reaction time at a dose of 1 mM, returning to the level of the control group at a concentration of 10 mM. 2PTS appears to modulate these immune cells, having generated safe concentrations of O_2^- at higher doses, without causing oxidative damage in canine PMNs. This study assumes the safety of using 2PTS in relatively low doses ([Chang et al., 2004](#)).

Cellular antiproliferative activity

DAS has been considered not only responsible for the modulation of the antioxidant enzyme system, but also an inhibitor of cell proliferation and inducer of tumor cell apoptosis (Afsheen et al., 2018; [Pantoja et al., 2000](#)). Also, the flavonoid apigenin contained in garlic, when hydroxylated, can inhibit tumor proliferation and angiogenesis ([Afsheen et al., 2018](#)). In a bioassay, it was evaluated whether there would be changes in the growth and metabolism of canine mammary tumor cells of lineage 13 (CMT-13) incubated with 6 OSCs. There was no significant change in the growth of cultures that were treated for 48 h with 1 mM of one of the three water-soluble compounds: S-allyl-cysteine (SAC), S-ethyl-cysteine (SEC) and S-propyl-cysteine (SPC) in relation to the exponential growth of the control culture. While of the three oil-soluble compounds, DAS and DADS presented cytostatic activities and DADS was the only one that presented cytotoxic activity. From these effects, it was deduced that the potency of these oil-soluble OSCs increases as the number of sulfur atoms increases. Exposure with 1 μ M DADS for 48 h substantially reduced the growth of CMT-13. An inverse relationship was also observed between the DADS concentration (0, 1, 10, 50, 500 or 1000 μ M) and the percentage of growth inhibition of CMT-13 ($r = -0.99$, $P < 0.05$). However, at concentrations of 500 μ M and above, DADS induced morphological changes in CMT-13, with some cells becoming rounded or rolled. Therefore, it was assumed that DADS influences the plasma membrane of these cells. The calculation of the effective dose of 50% DADS in

inhibiting the growth of CMT-13 found a concentration of 10.9 μM . The ratio between the DADS concentration (0 to 1000 μM) and the intracellular amount of glutathione was significantly related to the percentage inhibition of CMT-13 growth. Intracellular glutathione was dependent on the number of DADS added. There was no change in the growth of CMT-13 cells with the addition of 100 μM glutathione to the culture media. However, when the culture was pre-incubated with glutathione, the growth inhibition induced by 50 μM DADS decreased from 43% to 14%. There was no change in the growth of CMT-13 cells after pre-treatment with 50 μM DL-buthionine-SRsulfoxamine (BSO) for 24 h. However, this pre-treatment with BSO increased the growth suppression induced by DADS by 20%. These differences in the activity of garlic OSCs confirmed previous studies that observed a better action of crude ethanolic extracts of garlic than of aqueous extracts in inhibiting tumor cell growth ([Sundaram & Milner, 1993](#)). The safety of DADS was also observed due to its decreased action at higher doses.

In non-excitabile Madin-Darby canine kidney (MDCK) tubular cells, the addition of DAS (1-4 mM) dose-dependently induced a significant and sustained increase in cytosolic calcium concentration ($[\text{Ca}^{2+}]_i$) by phospholipase C-independent release of ionic calcium (Ca^{2+}) from the endoplasmic reticulum (ER) and by extracellular Ca^{2+} influx. It was also demonstrated that removal of extracellular Ca^{2+} inhibited the increase in $[\text{Ca}^{2+}]_i$ induced by DAS (4.0 mM) by 46% of the maximum value. Cell viability, after incubation with DAS (4 mM) for 24 h, showed 61.5% of MDCK cells with apoptotic DNA. DAS also induced cytotoxicity in renal cells independent of Ca^{2+} . In investigations of the effects of some OSCs on the viability of MDCK cells, it was suggested that the magnitude of apoptosis and cell death occurred in the following order: DAS < DADS < DATS. The antiproliferative effect of DAS may occur by modulating thiols in the cytoplasm and membrane, after the process is initiated by an increase in Ca^{2+} concentration. Cells exposed to sulfhydryl agents may alter their redox state, which deregulates $[\text{Ca}^{2+}]_i$, which in turn inactivates enzymes associated with cell function and growth ([Chen et al., 2009](#)).

Antimicrobial activity

The indiscriminate use of antibiotics without performing culture and antibiogram has generated multiple bacterial resistance in veterinary practice. Therefore, the exploration of new sources of natural compounds with antimicrobial activity is required. The antibacterial activity of different plant extracts can be influenced by their concentration, the choice of solvent and the extraction method ([Ionica et al., 2022](#)). Allicin has high antimicrobial activity and action against Gram-positive and negative bacteria by reacting with thiol groups of enzymes such as alcohol dehydrogenase, thioredoxin reductase and RNA polymerase ([Chavan et al., 2016](#)). Research has shown that the compounds allicin, ajoene, DADS and DATS have antibacterial properties ([Ezeorba et al., 2022](#); [Pantoja et al., 1991](#)). Garlic oil is commercially produced by collecting the vaporization of cooking at 100° C of the crude extract of powdered garlic (Mosallanejad et al., 2013), decomposing the unstable allicin in the oil fractions of DAS, DADS, DAT and Sandmethyl sulfide ([Ezeorba et al., 2022](#); [O'Gara et al., 2000](#)).

In a study involving 21 adult dogs infected with *Helicobacter* spp., two different treatments were administered. Seven dogs were treated with amoxicillin, metronidazole and omeprazole for 15 days, resulting in the eradication of *Helicobacter* spp., but there was no significant improvement in the parameters assessed by histopathological study. Another seven dogs were treated with garlic oil capsules (500 mg) for 30 days, presenting a significant reduction in the density of *Helicobacter* spp. and significant improvement in the following parameters assessed: inflammatory infiltrate and glandular degeneration of the gastric body region. Also, glandular degeneration of both the fundic and antral regions. No side effects were detected in the animals undergoing these treatments. This study confirms other studies, which show no correlation between the density of bacteria and the inflammatory parameters analyzed ([Costa et al., 2009](#)).

Salmonellosis is a zoonotic infection. Dogs and cats, in addition to being carriers, can develop a subclinical infection that can progress to mild to severe gastroenteritis, especially in immunosuppressed individuals. The antimicrobial effects of garlic extracts were tested with 19 strains of *Salmonella* spp. isolated from the feces of dogs incubated in Petri dishes with Mueller Hinton agar at 37°C for 24 hours. Three garlic extracts were used: ethanolic at concentrations of 100%, 50% and 25%; aqueous, using 70 g of fresh garlic; and oily (from Romania) containing 25 mg of allicin. In the bacteriological examination, the isolated *Salmonella* spp. were serologically classified into serological group B. The

reference used to measure the area of growth inhibition was the diameter of the *Salmonella enteritidis* ATCC 13076 strain in incubation with the extracts. The largest diameter of the growth inhibition area was observed in the incubation with 100% garlic ethanol extract and garlic oil, reaching approximately 30 mm. In the aqueous and oily extracts of garlic, the average diameters of the inhibition areas were similar. The diameter of the growth inhibition area with the aqueous extract ranged from 22 mm to 29 mm among the strains. While the smallest diameter of the inhibition area was observed in the 25% ethanol extract with approximately 11 mm. This study suggests in cases of salmonellosis with multiple resistance, the use of 100% garlic ethanol extract or garlic oil extract with a concentration of 25 mg of Allicin ([Ionica et al., 2022](#)). Since this work did not mention the number of garlic used in the ethanol extract, which is potentially toxic to dogs, further studies are needed.

Gingivitis is an inflammation with reversible hyperemia, initiated by the formation of bacterial plaque, which can progress to periodontitis. Halitosis is caused by bacterial degradation of volatile sulfur compounds (VSCs). *Corynebacterium* and *Actinomyces* species have been shown to hydrolyze N-benzoyl-DL-arginine-2-naphthylamide (BANA) in Beagles with periodontitis. The canine antimicrobial peptide cathelicidin (CAMP) is part of the innate immune response with a broad spectrum, directly modulating inflammation. In the treatment with 18 mg/kg/day of AGE (garlic from Japan) in 10 Beagles with mild gingivitis, there was an improvement in the following oral indicators evaluated: significantly reduced the gingival index score (0.53 ± 0.13 to 0.25 ± 0.08) in 2 months; suppressed the level of exhaled VSCs, which increased significantly in the control group after 2 months; there was a suppression of the hydrolytic activity of BANA only in the first 2 weeks; and significantly increased the salivary concentration of CAMP in relation to the control only in the first 2 weeks. This study assumes that the release of CAMP was induced by AGE; that salivary CAMP reduces the level of exhaled VSCs and the enzymatic activity of periodontal pathogens, consequently being able to partially suppress a discrete gingivitis in dogs. This work partially confirms the antioxidant, anti-inflammatory and immunomodulatory properties of AGE observed in other studies ([Takahashi et al., 2023](#)). Perhaps this low action of AGE occurred due to the variety or origin of garlic, the inadequate dose or the breed of dogs used.

Hypoglycemic activity

Diabetes mellitus (DM), either due to absolute or partial insulin deficiency, is the most common endocrine disorder of the canine pancreas. Several mechanisms control blood glucose levels within homeostatic limits. It is suspected that garlic may have a glucagon-lowering effect, lowering blood glucose levels. Garlic OSCs readily bind to cysteine compounds, which may prevent insulin inactivation by binding to its sulfhydryl group. Low-dose administration of the cytotoxic drug alloxone iatrogenic induction of diabetes due to partial insulin deficiency occurred in 21 adult dogs. Treatment with garlic tablets (100 mg/kg) for 14 days significantly reduced blood glucose levels compared with the hypoglycemic drug metformin hydrochloride (1700 mg/day) and the untreated diabetic group. Both treatments significantly increased serum insulin levels close to baseline. At the end of the experiment, diabetes was reversed in both treated groups ([Mosallanejad et al., 2013](#)). This study demonstrated that garlic tablets can reverse hyperglycemia in dogs.

Hypolipidemic activity

Among medicinal plants, garlic has a high serum cholesterol and triglyceride reduction in humans. Hyperlipidemia (increased serum triglyceride and/or cholesterol concentrations) in dogs is commonly secondary to conditions such as hypothyroidism, pancreatitis, cholestasis, nephropathies, obesity, and high dietary fat. In this clinical study, 15 male dogs aged 1 to 2 years were given cholesterol powder (4 g/kg/day) for 10 days to induce hyperlipidemia. The dogs were divided into 3 groups of 5 dogs. One group was treated with Atorvastatin (5 mg/kg) and the other group was treated with garlic tablets (100 mg/kg/Garlet 400mg: 1200 mcg of Allicin) for 45 days. Four blood samples were collected: on day 0 and day 10, before and after cholesterol supply, respectively; and on days 40 and 55 of treatment. Commercial kits were used to measure: total serum cholesterol, triglycerides, HDL-C and LDL-C. Atorvastatin ($p < 0.001$) and garlic ($P < 0.01$) presented activity hypolipemic in the reduction of serum triglycerides. Also, the Both treated groups reduced LDL-C at day 55 ($p < 0.001$). However, there was a significant increase in HDL-C in the Atorvastatin group compared to the garlic group on day 40 and in

the control group on day 55. With these data, Atorvastatin was considered more effective than garlic in improving the lipid profile. The antilipid effects of Atorvastatin occur through the competitive inhibition of -3-hydroxy-3-methylglutaryl-coenzyme A reductase (COA-HMG) in the initial biosynthesis of cholesterol. While the antilipid mechanism of action of garlic probably consists of reduced lipid absorption in the gastrointestinal tract and reduced synthesis of LDL-C. This study suggests that garlic may be used as an adjunct in the treatment of dogs with hyperlipidemia ([Mosallanejad et al., 2016](#)).

Antioxidant activity

Antioxidants slow down the oxidation process through several mechanisms: reduction of free radicals, chelation of metal ions or inhibition of enzymes. The flavonoid myricetin found in garlic neutralizes oxidative stress when generated by ROS. In an in vitro study, the freeze-dried methanolic garlic extract (from Faisalabad, Pakistan) showed high dose-dependent antioxidant potential even at very low concentrations (20 µg/mL) against the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). And the application of high concentrations (500/1000 µg/mL) of this freeze-dried extract also showed potential protection against DNA damage ([Afsheen et al., 2018](#)). This study demonstrated the antioxidant action of garlic in low concentrations.

Activity in activating the transition factor Nrf2

Nuclear factor erythroid 2 (Nrf2) modulates cellular responses by regulating the expression of the genes of the Phase II antioxidant and detoxifying enzymes (NFE2L2) against environmental stresses. Research indicates that both AGE and its compound SAC perform their functions through the signaling pathway of Kelch-like ECH-associated protein 1 (Keap1) that regulates Nrf2 activity (Keap1–Nrf2). SIPC is also suspected to be an inducer of Nrf2. This suggests that AGE may act as an inducer of Nrf2 and upregulate multiple genes related to antioxidation as well as detoxification through the Keap1-Nrf2 signaling pathway in dogs. In the treatment of 9 dogs with two doses of AGE (45/90 mg/kg) and water for 3 months, there was a significant increase in the expression of the Nrf2-regulated gene NFE2L2 after 2 months. This may have occurred by compensating for the reduction in cytoplasmic Nrf2 or by the action of AGE phytochemicals that upregulated this gene and the Keap1-Nrf2 signaling pathway in a synergistic manner. Among the four phase II antioxidant enzyme genes regulated by Nrf2, there was also a significant increase in gene expression: quinone dehydrogenase 1 (NQO1) in the second month and glutamate-cysteine ligase modifying subunit (GCLM) in the third month compared to the control. During the experiment there was no change in expression of the superoxide dismutase 2 (SOD2) gene with AGE administration. This study is assumed that AGE in relatively low doses can contribute to preserving the health of dogs ([Yamato et al., 2018](#)).

Activity against ectoparasites

More informed consumers are looking for non-toxic and natural alternatives to treat ectoparasites in their pets, because conventional insecticides develop resistance, putting your pet's health and yours at risk through direct contact. Several studies have demonstrated the insecticidal effects of garlic essential oils ([Abdel-Meguid et al., 2022](#)).

Volatile vegetable oil extracts are generally essential oils with a watery appearance, low viscosity and characteristic aroma. Garlic essential oil is composed of DADS (48.48%) and others, less than 10%, such as DAS, DATS, trisulfidemethyl-allyl, di-2propenyl, ajoene and allicin. A small proportion of terpenic compounds have also been isolated and identified in its essential oil ([Ezeorba et al., 2022](#)).

Pediculosis caused by the ectoparasite *Trichodectes canis* is a widespread infestation by chewing lice that feed on dead skin cells and sebaceous secretions worldwide. This louse is transmitted by direct contact and there are reports that it can serve as a mechanical vector for the tapeworm *Dipylidium caninum*. In the analysis by means of gas chromatography coupled with mass spectrometry (GC-MS), 45 phytocompounds were revealed in the essential oil of garlic (from a company in Cairo, Egypt), the most abundant being: 2,3,3-trimethylhexane (5.33%), tetracane (4.63%) and 4(Prop-2-enoyloxy) pentadecane (4.52%). An in vitro bioassay was performed to evaluate the toxicity of 400 µl of this essential oil of garlic at a concentration of 50% on the control of *Trichodectes canis*. The mortality rate (M%) was 100% from 15 minutes to 35 minutes of exposure. The sensitivity of *T. canis* to garlic oil was

demonstrated by the median lethal concentration (LC50) of 10.757% after 35 minutes. The relative efficacy of garlic oil after 35 minutes, using low-efficacy marjoram oil as a reference, was 2.5 times more effective. This study concluded that although garlic essential oil showed potential as an alternative control of *T. canis*, *in vivo* studies are necessary ([Abdel-Meguid et al., 2022](#)).

The toxicity of garlic and its phytochemicals in dogs

Any and all substances are potentially toxic depending on the dose. Food consumption is generally considered safe, but the presence of certain plants or their phytochemicals, can, depending on the dose or the enzymatic/metabolic system of a given species, act as phytochemicals ([Nagy et al., 2023](#)). Dogs suffer more poisoning accidents than cats because their eating habits are indiscriminate ([Cortinovis & Caloni, 2016](#)). Some reports have shown that garlic has a high potential for toxicity to dogs ([Hu et al., 2002](#)), by producing highly reactive oxidizing OSCs ([Nagy et al., 2023](#)). Moderate garlic intake by dogs is considered to be less toxic and safer than onion intake ([Kovalkovičová et al., 2009](#)), although a lower dose of cooked garlic extract (5g/kg) is sufficient to induce extravascular hemolysis ([Lee et al., 2000](#)), in relation to the considered toxic dose of onion (15-30g/kg) in dogs ([Kovalkovičová et al., 2009](#)).

Effect on the gastrointestinal system (GI)

Some compounds in raw or fresh garlic homogenates, such as allicin, which do not undergo changes in acidic pH ([O'Gara et al., 2000](#); [Yamato et al., 2018](#)), have the potential to cause irritation to both the skin and mucous membranes or may induce vomiting and diarrhea, and are not indicated in patients with gastritis ([O'Gara et al., 2000](#); [Takahashi et al., 2023](#); [Yamato et al., 2018](#)).

ASP in the presence of water generates allicin by activating alliinase. However, in one study, there was the isolation of a significant amount of alliin in the urine after ingestion of fresh garlic, evidencing the inhibition of the alliinase enzyme by the acidic pH of the stomach. Therefore, if the presentation format of the ASP mixture is in tablet or capsule form, the gastric pH inactivates alliinase, preventing the formation of allicin and its action ([O'Gara et al., 2000](#)). In toxicity tests in dogs, it was observed that, after oral administration of ASP, it caused severe inflammation in the gastric mucosa and, in tablet form, the loss of crypt cells in the ileum ([Hu et al., 2002](#)).

In the processing of the AGE extract through natural and enzymatic reactions, there is the elimination or modification of aromatic compounds and irritants contained in fresh garlic in CSOs stable, water soluble and less cytotoxic ([Amano et al., 2016](#)). There are reports that AGE causes less gastric irritation in dogs. However, in a study of 6 dogs treated with AGE for 3 months, 1-7 episodes of soft stools were temporarily observed in the group with half the dose of AGE (45 mg/kg) over the period, with one of the dogs having the highest incidence, which also had 4 episodes of vomiting. Since there was no increase in the inflammation marker (C-reactive protein), reduced appetite or weight loss in any of the dogs, this study assumed that AGE may cause a slight individual irritation in the GI tract of more sensitive dogs ([Yamato et al., 2018](#)).

Induction of extra and intravascular hemolysis

Garlic is toxic to erythrocytes of several domestic animals, particularly canines ([Chang et al., 2004](#)). Ingestion or administration of garlic extracts with many oxidant OSCs can induce extra- and intravascular hemolysis in dogs, either accidentally or experimentally ([Chang et al., 2005](#); [Hu et al., 2002](#); [Yamato et al., 2018](#)), when their levels exceed the antioxidant capacity of erythrocytes ([Salgado et al., 2011](#)). Case reports in dogs about the ingestion of food with *Allium* plants, regardless of the form of preparation, generated toxicity either by single exposure to a large dose or by repeated exposure to smaller toxic doses due to its cumulative effect on erythrocyte oxidation ([Kovalkovičová et al., 2009](#)).

Oxidation is part of cellular metabolism that leads to the formation of free radicals such as reactive oxygen species (ROS) ([Afsheen et al., 2018](#); [Ezeorba et al., 2022](#)), which are important in intercellular signaling and energy production ([Afsheen et al., 2018](#)). However, overproduction of ROS leads to oxidation of macromolecules and activation of degeneration ([Afsheen et al., 2018](#); [Ezeorba et al., 2022](#)). Canine erythrocytes are naturally predisposed to oxidative damage due to the low antioxidant activity of the catalase enzyme ([Salgado et al., 2011](#)). Oxidants can cause direct injury to the membranes and

the cytoskeleton erythrocyte, which can cause adhesion of opposing areas of the membranes, resulting in the formation of rigid spheroids, the centrocytes ([Chang et al., 2005](#); [Hu et al., 2002](#); [Lee et al., 2000](#)).

Evidence suggests that bio-compounds derived from allicin may be responsible for oxidation of iron from the ferrous state (Fe^{2+}) to ferric (Fe^{3+}) in the reversible formation of methemoglobin (Met-Hb) ([Chang et al., 2005](#); [Ezeorba et al., 2022](#)). The precipitation and denaturation of Met-Hb molecules results in the formation of Heinz bodies within erythrocytes ([Yamato et al., 2003](#)). The formation of malondialdehyde by lipid peroxidation together with O^{2-} denatures hemoglobin by oxidation of sulfhydryl groups and β -93 cysteine residues (sulfhemoglobin), precipitating hemoglobin that binds to the cell membrane to form Heinz bodies ([Salgado et al., 2011](#)). Successive oxidation promotes the growth of Heinz bodies by condensation, deforming and distorting the cell plasma membrane ([Lee et al., 2000](#)).

Erythrocytes with Heinz bodies and erythrocytes, because they are less flexible ([Nagy et al., 2023](#)), tend to be sequestered, perforated or phagocytosed by the mononuclear phagocytic system, accelerating extravascular hemolysis ([Lee et al., 2000](#); [Yamato et al., 2005](#)). The perforated cells become spheroids, increasing their osmotic fragility, which results in intravascular hemolysis ([Yamato et al., 2005](#)). Therefore, these erythrocyte oxidations can cause hemolysis.

In some dogs, due to genetic inheritance, their erythrocytes are more susceptible to oxidants: some individuals of the Japanese (Shiba inu and Akita inu) and Korean (Jindo) breeds, paradoxically, have high concentrations of GSH and potassium ([Yamato et al., 1999, 2005, 2018](#)) and uncertain ethnic groups because they present deficiency of glucose-6-phosphate dehydrogenase (G6PD) activity due to a metabolic defect, interfering with GSH regeneration through the pentose phosphate pathway, increasing O^{2-} levels ([Salgado et al., 2011](#); [Yamato et al., 1999](#)). Some dogs may also be more susceptible to oxidants due to nutritional zinc deficiency ([Nagy et al., 2023](#)) by interactions with dietary factors or medications (propofol, propylene glycol, dl-methionine, sulfonamides, sulfapyridine, high doses of vitamin K3 or benzocaine) ([Salgado et al., 2011](#)) or by presenting diseases associated with oxidative stress ([Lee et al., 2000](#)). In these susceptible dogs, oxidative injury is more serious, may develop hemolytic anemia by providing foods containing garlic ([Lee et al., 2000](#)).

A case of hemolytic anemia was reported for the first time in an adult Schnauzer dog induced by the consumption of a certain number of cooked garlic and Chinese chives, which after 2 days presented anorexia and dark urine. On admission, the dog presented mild hemolytic anemia, neutrophilia with a left shift, the presence of eccentrocytes (37.5%) and erythrocytes with Heinz bodies. The anemia progressively increased with the hematocrit (Ht) reaching a nadir (19%) on the fourth day, with the percentage of eccentrocytes (21.6%), erythrocytes with Heinz bodies (44.3%) and Met-Hb concentration (2.1%) remaining high. Persistent leukocytosis and thrombocytopenia were also observed. It was assumed that Chinese chives, because they have a flavor similar to other *Allium* plants, may also contain oxidants. The genetic susceptibility to oxidative stress in this dog was investigated and ruled out, with improvement only beginning on the ninth day ([Yamato et al., 2005](#)).

A case of hemolytic anemia associated with systemic hypertension (182 mmHg) was also reported for the first time in an adult Schnauzer dog induced by the accidental ingestion of approximately 60 g of roasted garlic, which after 2 days, presented vomiting and dark urination. On admission, the patient presented regenerative anemia with Ht of 22%, stress leukogram with moderate leukocytosis and thrombocytosis. Blood analysis showed occasional eccentrocytes, Heinz bodies, Met-Hb and hemolysis (8.7%). He also presented hypokalemia and elevated concentrations of several hepatobiliary enzymes. Urinalysis revealed a specific gravity of 1.008, proteinuria and hematuria. Other conditions that could cause hypertension in dogs were ruled out. Severe hemolysis can cause glomerulonephritis and Met-Hb induces hypoxia that leads to peripheral vasoconstriction, worsening renal ischemia and possibly causing hypertension. The dog was discharged after 4 days of hospitalization, but the hypertension was only corrected after 4 months of treatment ([Kang & Park, 2010](#)).

Preclinical trials and clinical

When 5 g/kg of water-soluble cooked garlic extract (from Korea) was administered to 4 dogs for 7 days, there was a significant decrease in the erythrocyte count and hematocrit concentration on the 8th

day, reaching a minimum on the 10th day after treatment. After that, the erythrocyte counts gradually increased without reaching the initial value. There was also a significant neutrophilia on the 7th and 10th day of treatment compared to the control. There was a slight non-significant increase in the mean Met-Hb concentration on the 4th day of treatment. The mean percentage of erythrocytes with Heinz bodies increased significantly on the 5th day and reached a maximum (6.91%) on the 9th day, then gradually decreased. There was a significant increase in the percentage of eccentrocytes on the 7th day, reaching a maximum ($15.52 \pm 12.24\%$) on the 8th day. The concentration of GSH in erythrocytes increased significantly on the 6th day, returning to the initial value on the 31st day. These changes were not found in the control group. Intravascular hemolysis or hemoglobinuria was not observed in any of the dogs (Lee et al., 2000). Therefore, an extravascular hemolytic anemia was developing sub clinically, which, through its cumulative effect, could cause intravascular hemolysis. This indicates that the administration of cooked garlic extract (5 g/kg) to dogs for 7 days induced, through the reductions in the hemogram and the oxidative lesions found, extravascular hemolysis.

In the fractionation of the aqueous extraction of ethyl acetate (EtOAc) from garlic (imported from China), 5 volatile oily OSCs were isolated and identified, followed by 3 more colorless oily OSCs: a mixture of three symmetrical sulfides of DATS [1], tetra- [2] and pentasulfide diallyl [3]; diallyl thiosulfonate [4] and trans-sulfuric-3-allyl-sulfanyl-allylic acid allyl ester [5] (9); propenyl-allyl thiosulfinate [6], 2-propene-1-sulfinothioic acid S-(E)-1-propenyl ester [7] and the volatile trans-sulfuric-3-allyl-sulfanyl-allylic acid allyl ester [8] (Yang et al., 2003). In the bioassay to evaluate the oxidative activity in canine erythrocytes in a dose-dependent manner, the mixture of compounds 1-3 showed a higher oxidative activity than compounds 4 and 5 (Hu et al., 2002) and compounds 7 and 8 showed a much higher oxidative activity than compound 6 (Yang et al., 2003). Furthermore, the separation of the extract layers, both the ethyl acetate and the aqueous layers, equivalent to 5 g of garlic, showed a concentration (47%) of Met-Hb higher than that of its isolated compounds, indicating by these studies the existence of other unidentified oxidants in the extracts (Hu et al., 2002, Yang et al., 2003).

In vitro analysis of the oxidative activity of the natural phytochemical 2PTS present in cooked garlic in canine erythrocytes showed a significant increase in the concentration of Met-Hb, the percentage of erythrocytes with Heinz bodies and the turbidity index (quantitative estimate of Heinz bodies) (Yamato et al., 2003). This bioassay confirms the experiment by Lee et al. (2000) that phytochemicals in cooked garlic have the potential to induce hemolysis in dogs.

In a study of 9 dogs treated orally with AGE for 3 months, there was only a significant increase ($p < 0.05$) in the reticulocyte count in the group with half the dose (45 mg/kg) compared to the control in the first week, and no oxidative markers were found in the blood smear (Yamato et al., 2018). In the regenerative response, the release of young erythrocytes is accompanied by an increase in GSH concentration and in the activity of enzymes associated with its metabolism (Lee et al., 2000). Would a lower dose induce an antioxidant response?

Conclusion

There has not yet been standardization regarding the variety of *Allium sativum*; the origin of the plant with the best proportions of bioactive phytochemicals; and the extraction or preparation methods with their variables that guarantee less loss and selectivity of their active compounds. Further *in vivo* and toxicity studies are needed to prepare dog-specific herbal formulations with relatively low doses of the active phytoconstituents of garlic. Regardless of the method of preparation, dog owners should be advised that it is not safe to provide or add garlic to their dog's food.

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