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Allium cepa bulbs (Alliaceae) and *Syzygium aromaticum* spices (Myrtaceae) aqueous extracts mixture protects the knee joint and the features associated with movement in a model of locomotor dysfunction induced by the monoidoacetate on Wistar rat

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Abstract

Background: *Allium cepa* (Onion) is widely used as a flavored vegetable in various types of foods and against urinary system disorders, ear disorders, and injuries. *Syzygium Aromaticum* (clove) is used as a local anesthetic, also used against pain, rheumatological, and antioxidant problems. This study aimed to show the protective effects of the aqueous extract of the mixture of Onion and clove in the occurrence of a joint pathology such as osteoarthrosis.

Methods: The animals were previously treated for two weeks with the aqueous extract (100 and 200 mg/kg) with the mixture of *Allium cepa* bulbs and *Syzygium aromaticum spices,* osteoarthrosis was induced with monoiodacetate (50 µL, intraarticular). Clinical, oxidative stress, inflammation, hematological and histological parameters were evaluated.

Results: The mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices (200 mg/kg) protected the animals against the increase of sensitivity (80.78 %) and the swelling in the paw (85.27 %). Pre-treatment with the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices has significantly protected animals by improving their locomotion. This mixture has prevented the negative variation of hematological parameters. Antioxidant effects were observed with an increase in SOD, CAT activity, GSH concentration, and reduction of MDA, NO levels. The mixture administered orally, preventively showed an anti-inflammatory effect by significantly reducing TNF-α, IL-1β, MMP9 concentrations, and MPO activity. In animals pretreated with the mixture, the knee joint appears healthy, thus showing a protective effect of the extract.

Conclusion: The mixture of *Allium cepa* bulbs and *Aromaticum syzygium* spices is a constituent worthy of more in-depth study to determine whether it effectively modulates the complex inflammatory pathology of osteoarthritis according to its multiple multi-component actions.

Keywords: *Allium cepa*; *Syzygium aromaticum*; locomotion; monoiodacetate.

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Background

Osteoarthritis, degenerative joint arthropathy with evolutionary loss of joint cartilage, is a very widespread musculoskeletal pathology in the world leading to an important burden for health systems in general and for individuals in particular. When the carrying joints (hips and knees) are affected, reduced mobility, joint pain and stiffness of the joint are observed. Osteoarthritis is also considered as an autoimmune disorder given that during its development, the immune system attacks mucous membranes which surround the joints (the synovial), thus causing pain, inflammation and joint lesions [1]. During its evolution which is a function of time and/or age, synovial thickening is observed in affected people, subchondral bone alterations, articular cartilage damage, osteophytes formation and joint loss of architecture. Osteoarthritis is a multifactorial disease involving hereditary alternations, genetic, endocrine or metabolic predisposition, mechanical stress, obesity, joint overload, trauma and prolonged exposure to chronic inflammation [2,3].

Inflammation, chronic pain, anxiety and oxidative stress are an integral part of the feeling of people with osteoarthritis. Indeed, the concentrations of cytokines (Interleukin 6 (IL-6), Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β)), chemokines as well as prostaglandins were found very high in people with osteoarthritis [2]. It is known that a localized inflammatory response, a significant mechanical load and aging can lead to a significant increase in the production of reactive species of oxygen (ROS), nitric oxide (NO), superoxide anion, peroxinitrite, hydrogen peroxide (H_2O_2), lipid peroxidation, alterations of cell signaling damages to nuclear deoxyribonucleic acid (DNA) [4]. This increase in oxidative stress will cause the articulation of anomalies in the metabolism of bones and cartilage at the level of the metabolism with the consequence of their degradation and also the overall disturbance of the repairing potential of osteoblasts, chondrocytes and/or their precursors. Thus, antioxidant therapy that improves enzymes activities such as Catalase (CAT), reductase glutathione (GSH), superoxide dismutase (SOD) and/or peroxidase can be beneficial for patients with osteoarthritis [5]. It is known that people who suffer from osteoarthritis, in addition to chronic pain, continuous stiffness, repeated falls and knowing that they impose an important economic burden, they very often develop mental health problems (depression, stress, anxiety and alteration of quality of life). In addition, 40 % of patients with osteoarthritis of the lower limbs have an anxiety or depression state [6].

Knowledge of the etiology of osteoarthritis these days requires an urgent need for effective therapies that will alleviate symptoms but also improve the well-being of people affected by osteoarthritis. Currently, no sufficiently effective treatment has been found against osteoarthritis, since existing treatments control symptoms, reduce pain, but are limited in efficiency and are also associated with toxicity [7,8]. Therapeutic approaches to the treatment of osteoarthritis are limited because no drug is available, disease and treatment with analgesic compounds has reduced effectiveness and significant side effects [9]. Consequently, the development of effective modifying drugs and new therapeutic strategies according to the osteoarthritis phenotypes is urgent [10,11]. Management of osteoarthritis can have a beneficial role in reducing inflammation, degradation of cartilage and/or bones and considerably reduce oxidative stress. For example, for 40 years, a phytotherapeutic, non-toxic, although slow and requiring long-term therapy, has been administered to the osteoarthritis patient in two forms (topical and compressed) [12].

Allium cepa (Alliaceae family) and *Syzygium aromaticum* (myrtaceae family) very well known as the onion and clove respectively have the origin of Central Asia (onion) [13], then Indonesia, Philippines, Moluccas Islands, Africa and South America [14]. *Allium cepa* (*A. cepa)* is perennial grass so all parts (plant, leaves, and flowers) are used for various purposes. The onion is widely used as flavored vegetables for its aroma and taste in various types of foods in the world. Its therapeutic and medicinal values are also known, most of which are scientifically validated. In the northern part of Cameroon, the onion is widely cultivated and consumed in cooked or raw form. Consumed as vegetables, it is also used as a main source of flavor, condiments, medicines and is rich in carbohydrates, proteins, fats, vitamins, minerals, sugars, fibers, saponin, tannin, etc. [15,16]. At the traditional level, it is used against metabolic disorders, urinary system disorders, pneumonia, sexual disability, skin diseases and ear disorders and injuries [16]. At the scientific level, its pharmacological properties are numerous, namely: antioxidants, anti-platelets, anti-inflammatory, analgesic, antibiotic, immunoprotector, anti-diabetics, antimicrobials, hypolipidemia and many others [17,18,19]. This plant contains flavonoids, glycosides, terpenoids, saponins, alkaloids, tannins and anthocyanins [20,21]. The presence of components such as quercetin and kaempferol has been demonstrated [22,23]. *Syzygium aromaticum* (*S. aromaticum)* is a large tree of an average height of 10 to 12 m, which can reach up to 20 m high, with a light gray trunk. Its leaves, 8 to 10 cm long, are tough, persistent, opposite, petiolate, oval, with lanceolate limbo, on the upper reddish green and on the lower dark green, slightly punctuated [24]. This plant contains the flavonoids, tannins, phenolic acid, sterols, triterpenes, eugenol, eugenol acetate, alpha- and betacaryophyllene and a ketonic derivative [25]. Traditionally, it is used as a local anesthetic (for dental pain), also used against pain, rheumatological problems, such as antibacterial, antioxidant, antifungal and local anesthetic in wound care [14,25]. Previous studies have shown that this plant has antibacterial, antioxidant, antiviral and analgesic properties [24, 25]. In Cameroon, it is used in traditional pharmacopoeia as antiseptic, antispasmodic and used against digestive and renal disorders [26]. Used separately, *A. cepa* and *S. aromaticum* have significant pharmacological properties on the one hand, and on the other hand are consumed by many people for their nutritional power. Well, having each of the anti-inflammatory properties, no scientific study to date has demonstrated the importance of the food supplement of these two foods in the prevention of osteoarthritis. The food supplement of the *A. cepa* and *S. aromaticum* mixture could modulate the specific targets of osteoarthritis and other molecular targets involved in hypersensitivity mechanisms and even prevent neuronal lesions in the osteoarthritis joints linked to the severity of the disease. Thus, the aim of this study was to show the protective effects of the aqueous extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices in the occurrence of a joint pathology such as osteoarthrosis.

Methods

Preparation of plant materials

A. cepa bulbs and *S. aromaticum* spices were bought at the central market in Douala (Douala, Littoral Region, Cameroon) in May 2024. *A. cepa* and *S. Aromaticum* have been identified by authentication at the National Herbarium of Cameroon where the samples are preserved under the numbers 42791/HNC for *A. cepa*

and 66995/HNC for *S. aromaticum*. After having carefully and separately washed, cleaned, cut (small pieces), dried (sheltered from the sun) and crushed (reel) the *A. cepa* bulbs (Alliaceae) and *S. aromaticum* spices, a powder was obtained for each plant. Five hundred (500) g of each powder were macerated separately in 3 liters of distilled water for 24 hours then filtered (Wattman paper n°4), the residues obtained were still macerated a second time in 2 liters of distilled water for 24 hours and filtered (Wattman paper n°4) again. Each filtrate was dried in the oven (40 °C), which made it possible to obtain 28.5 g of extract from *A. cepa* and 23.6 g of *S. aromaticum* extract for respective yields of 5.7 % and 4.72 %. The mixtures administered to animals were in the proportions 50/50.

Chemicals

The Mono-iodoacetate (MIA) was provided by Markal-Za (France). The analysis kits for TNF-α, IL-1β, myeloperoxidase (MPO), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Creatinine and matrix metaloprotein-9 (MMP-9) for Immuno-enzymatic Rat tests were bought from Sigma-Aldrich (St. Louis, MO, USA). The Ellman and Griess reagents were provided by Sigma-Aldrich and freshly prepared before use. The Diclofenac was obtained in a pharmacy in the square. Each solution has been prepared fresh and administered per bone in a volume of 10 mL/kg of body weight.

Animals

Wistar rats (males and females), aged three months (weighing on average 165 g) were raised (laboratory pet store of the University of Yaoundé I, Cameroon's) under the standard light (day/night cycle of 12 hours), with access to the standard diet and water Ad Libitum during the test. The animals were housed in groups of 3 rats per cage (40 cm × 40 cm). All experiences followed the principles of the use and care of laboratory animals of "directives of the European Community" (ECE Directive 2010/63/EEC) and were approved by the "Institutional Ethics Committee for Research on Human Health" of the University of Douala (N°4729 CEI-UDo/01/2024/T).

Animal treatment

Thirty (30) rats were distributed according to their body mass in 5 cages of 6 rats each. The animals of cage 1, considered as normal control, received Sodium chloride (NaCl) (0.9%, 10 mL/kg), the animals of the cage 2, a negative control, also received NaCl (0.9%, 10 mL/kg), the animals of cage 3, positive control, received the diclofenac (5 mg/kg, 10 mL/kg) and the animals of cages 4 and 5, treated groups, received the aqueous extract from the mixture of *A. cepa* bulbs and *S. aromaticum* spices at the respective doses of 100 mg/kg (10 mL/kg) and 200 mg/kg (10 mL/kg). All treatments were administered daily and orally for 34 days using an esophageal probe.

Osteoarthritis induction

On the 15th day, all animals were anesthetized by intraperitoneal injection of diazepam (0.1 mg/kg) and ketamine (0.2 mg/kg). After anesthesia, the left knee of each animal was disinfected with iodized alcohol (yellow betadine). Using an insulin syringe, 50 µL of NaCl (0.9 %) was administered in the knee of each animal of cage 1 (normal control) and 50 µL of MIA (100 mg/kg) diluted in NaCl 0.9 % was administered in the knee of each animal of cages 2, 3, 4 and 5.

Clinical parameters assessment

Body mass

The body mass of animals was measured every day using an electronic rat balance.

Assessment of the anti-hyperalgesia effect of the mixture of A. cepa bulbs and S. aromaticum spices

The anti-hyperalgesia effect was evaluated using a heating plate (Ugo, Italy, Basile). For this effect, each animal was placed on the heating surface, the weather was launched and as soon as the animal licked and/or lifted the paw, the time was stopped. To avoid tissue lesions in animals, maximum stimulation time of 20 seconds was used in the event of non-reaction of the animal. For each rat, the test was carried out on day 0 (before the start of treatment), then the days 7, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 and 34.

Assessment of the anti-inflammatory effect of the mixture of A. cepa bulbs and S. aromaticum spices

The anti-inflammatory effect was measured using a water plethysmometer. To do this, each animal's paw was plunged into a 10 ml graduated burette containing water. When the water reached the knee of the animal, the quantity of water (mL) moved to the burette was in function of the volume of the soaked paw and informed about the thickness of the paw. This measure was made on days 0 (before the start of treatment), then on days 15, 17, 19, 21, 23, 25, 27, 29, 31 and 33.

Assessment of the activity of the mixture of A. cepa bulbs and S. aromaticum spices on the locomotion disorders

To observe the behavior and the locomotion of animals and why animal activity is very intense in the night, this test was carried out in the night (between 8:00 p.m. and 11:00 p.m.) in a calm environment, before the start of any treatment, then once a week [27] using an Open Field Test. This open field was made of wood (80 cm in length × 80 cm in width × 60 cm high) with a background divided into small tiles of 0.04 m^2. Each animal was caught by the tail and placed in the center of the device and its displacement was recorded using Any-Mayse software for a period of 5 minutes. The parameters evaluated were the state of the stool, jumping number, licking, total distance travelled, average speed, total mobility time, total immobility time, mobility time episode, Immobility time episode, time in the peripheral zone, distance traveled in the peripheral zone, number of entries to the central zone and time in the central zone. All rat movements were automatically recorded and analyzed by a 7.15 video system (Stoelting, Wood Dale, IL, USA) with a high-resolution Logitech camera [28,20].

Sampling

On the 34th day, all the animals were anesthetized (thiopental, 50 mg/kg, *i.p.*), then sacrificed. The blood was taken by catheterization of the abdominal aorta and collected in two tubes (dry and with EDTA (Ethylene diamine tetra acetic acid)). The EDTA tubes were used for the blood formula count, while the dry tubes were centrifuged (2500 rpm, 15 minutes), and the supernatant was taken for biochemical analysis (ALT, AST, creatinine and proteins). The synovial fluid was taken by washing the joint (100 µL of 0.9% sterile NaCl) for determining the level of

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pro-inflammatory cytokines (TNF-α and IL-1β), of MMP-9 and MPO. Parts of the liver, kidney, brain and joint were crushed separately in sorted buffer (10 mm, 4 °C, pH 7.4) cold at 10%, centrifuged (10,000 rpm, 4 °C, 15 minutes) and kept at -20 °C. The different homogenates were used for the evaluation of the oxidative stress parameters (GSH, SOD, Malondialdehyde (MDA), Catalase, nitric oxide (NO)). The joints were fixed in 10% formalin for the creation of histological cuts.

Histological analysis

The knee joint set in 10 % formalin was used for routine coloring. It was dehydrated in alcohol and integrated into paraffin blocks, then 5 µm thick cuts were colored with hematoxylin and eosin (H&E). Colorful and climbed slides were observed using a scientific STM-50 optical microscope (HSIDC Industrial Estate, Haryana, India) equipped with a Celestron 44421 digital camera connected to a computer (HP Pavilion, G series).

Statistical analysis

The results of this study, presented as average ± ESM (standard error of mean), were analyzed using Graph Pad Prism (version 8.00; Graph Pad Software, Inc., San Diego, CA, USA) using a unidirectional variance analysis (ANOVA) followed by the Tukey-Kramer multiple comparison test; Then a multidirectional variance analysis (ANOVA) followed by the multiple comparison test of Bonforoni.

Results

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on body mass, hyperalgesia and inflammation

In Figure 1, the results show that body mass increases in all experimental animals during the first two weeks of treatment. After administration of the MIA, body mass decreases in animals of the negative control group compared to animals of the normal control group, this decrease is significant (p<0.01, p<0.001) from day 20 until the end of the experiment. The extract from the mixture of *A. cepa* bulbs and *S. aromaticum* spices significantly protected animals (100 and 200 mg/kg) against loss of body mass and this from the 22nd day to the end of the treatments.

The injection of MIA in the knee of animals has caused a significant increase (p<0.001) of pain sensitivity and the volume of the paw from the 15th day in animals of the negative control group compared to the animals of the normal control group which has remained significant throughout the experiment. The aqueous extract of the mixture of *A. cepa* bulbs and the *S. aromaticum* spices protected animals against the increase in the sensitivity and volume of the paw. The maximum effects were 80.78 % (200 mg/kg) for 32nd day against hypersensitivity and 85.27 % (200 mg/kg) for the volume of the paw on the 15th day. The effect of the extract was greater than that of diclofenac which showed a maximum effect of 22.54 % on the 28th day against pain and 29.88 % on 19th day for inflammation (Figure 1).

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on the number of stools, jumps and licking

It emerges from figure 2 which shows the numbers of stools, jumps and licking that all these parameters were normal and did not vary in all animals during the first two weeks. After injection of MIA, there is a significant increase in the number of stools, the number of licking and a significant decrease in the number of jumps in animals of the negative control group compared to the animals of the normal control group. The aqueous extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices has significantly protected the animals treated by showing in the latter the significant decrease in the number of stools licking and the significant increase in the number of jumps compared to the animals of the negative control group.

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on anxiety and spontaneous locomotion

Before the injection of MIA in all animals, the parameters such as the total distance travelled, average speed, total mobility time, total immobility time, mobility time episode, immobility time episode, number of lines crossing, time spent in the peripheral zone, time spent in the central zone, distance travelled in the peripheral zone and number of entry into the central zone were normal in the central area in the first weeks of treatment (Figure 3 and 4). After injection of MIA, all these parameters were affected in animals of the negative control group with a decrease in the total distance traveled, average speed, total mobility time, mobility time episode, number of lines crossing, time spent in the central zone followed by an increase in the total immobility time, immobility time episode, time spent in the peripheral zone, distance traveled in the peripheral zone and number of entry into the central zone. Preventive treatment based on the extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices has significantly protected the animals treated by improving their locomotion which was close to that of the animals of the normal control group (Figure 3 and 4). These results are confirmed by Figure 5 which clearly shows the image of the different displacements of animals in the open field.

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on some hematological parameters of arthritis rats

The levels of white blood cells, platelets, lymphocytes, monocytes, granulocytes and average hemoglobin corpuscular concentration have significantly increased while the levels of red blood cells, hemoglobin and average corpuscle content of hemoglobin have significantly decreased in non-treated osteoarthritis (Table 1). The aqueous extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices significantly protected the variation of hematological parameters by preventing a significant decrease in red blood cells, hemoglobin and average corpuscle content of hemoglobin and a significant increase in white blood cells, platelets, lymphocytes, monocytes, monocytes, monocytes granulocytes and average hemoglobin corpuscular concentration.

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on some parameters of liver and renal functions

Table 2 shows that the injection of MIA has caused a significant serum increase in ALT, AST and creatinine levels, then a significant decrease in protein levels in animals in the negative control group. The oral administration of the extract from the mixture of *A. cepa* bulbs and *S. aromaticum* spices has protected hepatic and renal functions by significantly reducing serum, ALT,

AST activity and creatinine levels and increasing serum protein concentration.

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on some oxidative stress parameters

In animals of the negative control group, we observe in the brain, the liver, the kidneys and the joint, a significant decrease in the activities of SOD, CAT and the concentration of the GSH, followed by a significant increase in MDA and NO compared to the animals of the normal control group. The aqueous extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices (100 and 200 mg/kg) showed an important antioxidant effect by significantly increasing the activities of SOD, CAT and the concentration of GSH, then significantly reducing MDA and NO levels (Figure 6).

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on some inflammatory parameters

The concentrations of TNF-α, IL-1β, MMP-9 and the activity of the MPO of osteoarthritis animals are illustrated in Figure 7. It turns out that the injection of MIA has led to an increase in the levels of TNF-α, IL-1β, MMP-9 and the activity of MPO in the synovial liquid of non-treated osteoarthritis animals. The aqueous extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices (100 and 200 mg/kg) administered orally and preventively showed an anti-inflammatory effect by significantly reducing the concentrations of TNF-α, IL-1β, MMP-9 and the activity of MPO.

Effects of the aqueous extract of mixture of A. cepa bulbs and S. aromaticum spices on the microarchitecture of the knee joint of arthritis rats

The knee joint of the rats of the normal control group has normal architecture showing all the well-illustrated parts (Figure 8). In non-treated osteoarthritis animals, the joint has leukocyte infiltration granulomas and synovial hyperplasia. In animals treated preventively with the aqueous extract of the mixture of *A. cepa* bulbs and *S. aromaticum* spices, the knee joint appears healthy thereby showing a protective effect of the extract.

Discussion

This study was carried out in order to assess the protective effects of the mixture of *A. cepa* bulbs and *S aromaticum* spices against the occurrence of osteoarthritis. For this, osteoarthritis has been induced in *Wistar* rat by intraarticular injection of MIA. The aqueous extract of the mixture of *A. cepa* bulbs and *S aromaticum* spices was administered orally to the doses of 100 and 200 mg/kg. Treatment began two weeks before osteoarthritis induction.

The MIA induced osteoarthritis model is very similar to the histological behavior and pain of human degenerative osteoarthritis [30]. Once the MIA injected into the rat's joint, there is a reduction in the number of chondrocytes, increased destruction of cartilage, as well as the decrease in proteoglycan synthesis and death cellular, thus showing histological and morphological articular alterations similar to human osteoarthritis modifications [30,31]. Many studies have shown the role of oxidative stress and inflammation in the progression of osteoarthritis [32,33]. In addition, these molecular mechanisms are involved in peripheral and central awareness underlying chronic pain which represents its main clinical manifestation [34,35]. In the early stages of osteoarthritis, pain is generally limited to the affected joint, but this pain can

spread in several regions of our body. Consequently, according to clinical results, patients with osteoarthritis suffer from secondary hyperalgesia due to the hypersensitivity of pain by central neuronal plasticity [35]. These clinical symptoms are generally followed by patient disability characterized by a reduction in mobility and movements. In this study, MIA's intra-articular injection has caused significant hypersensitivity, chronic inflammation and a reduction in the movement's capacity for all non-treated osteoarthritis animals. The preventive administration of the mixture of *A. cepa* bulbs and *S. aromaticum* spices has significantly protected animals treated against hypersensibility, development of inflammation and against functional disability. It is important to note that the combination of pharmacotherapy remains a useful and important strategy in the context of the multifactorial mechanisms of chronic pain. It is known that several natural derivatives products display very beneficial synergistic interactions for patients [36]. The analgesic, anti-inflammatory and protective effects of the significant movement capacity of the mixture of *A. cepa* bulbs and *S. aromaticum* spices in this study could, at least in part, be linked to its anti-inflammatory and antioxidant capacities.

Osteoarthritis is a pathology with a multifactorial etiology [37]. Its pathophysiology, which is very complex implies mechano-transduction, an interaction between protease inhibitors, metalloproteases and cytokines on the degradation of cartilage. Many cytokines (TNF-α, IL-1β, IL-6) are very involved in the progressive degradation of the tissue of joint cartilage [38,39]. Monocytes, which produce high levels, promote the synovitis process and osteoarthritis pathogenesis [40]. In this study, the mixture of *A. cepa* bulbs and *S. aromaticum* spices exercise powerful analgesic and anti-inflammatory activities, thanks to several mechanisms of IL-1β, IL-6 and TNF-α regulation [41,42]. Oxidative stress and inflammation contribute significantly to the development and progression of the pathology of osteoarthritis, in particular the lesion of cartilage, articular inflammation and bone remodeling, by disturbing the survival of chondrocytes, damaging the matrix and the promotion of the production of pro-inflammatory mediators such as various metalloprotein and interleukins [43]. Therefore, the inflammatory cytokines (IL-1, IL-6, TNF-α) play a pivotal role in the inflammation process of induced osteoarthritis, which makes them promising therapeutic targets [44]. Indeed, several clinical studies have shown that increased levels of inflammatory cytokines in the elderly are strongly associated with increased morbidity and mortality in age -related diseases, including osteoarthritis [45,46]. Despite this quite established understanding of pathology osteoarthritis, considering inflammation and inflammatory cytokines as potential therapeutic targets, no single inhibitor against these targets has shown proven clinical efficiency. This is probably due to the fact that the inflammatory process in osteoarthritis is very complex which involves multiple inflammatory mediators and signaling pathways [47]. In this perspective, it is conceivable that the significant relief of the symptoms of osteoarthritis such as pain, inflammation, destruction of cartilage and functional handicap presented in the mixture *A. cepa* bulbs and *S. aromaticum* spices, are based on multi-pharmacological anti-inflammatory activity against several targets. To further test this secondary hypothesis, the activity of mixture of *A. cepa* bulbs and *S. aromaticum* spices against several pro-inflammatory mediators was examined in this study.

The mixture of *A. cepa* bulbs and *S. aromaticum* spices significantly inhibited MPO, MMP-9, IL-1β and TNF-α in synovial fluid in animals after induction of MIA osteoarthritis. IL-1β, which accelerates the degradation of cartilage by increasing the expression and activity of enzymes degrading the matrix while interfering with the synthesis of the main components of

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extracellular cartilage matrix, is one of the most important inflammatory and catabolic cytokines in the pathophysiology of arthritis [48]. MMPs are major factors contributing to the degenerative pathology of osteoarthritis and as first level therapeutic targets so that drugs control osteoarthritis of various etiologies by their inhibition [49]. Among several MMPs, MMP-9 is involved in the senescence of chondrocytes induced by mechanical overload in osteoarthritis [49,50]. In osteoarthritis cartilage and synovial fabric, the MMP-9 is an enzyme which is increased by IL-1β and TNF-α, and it contributes significantly to the degradation of collagen. The fact that MMP-9 is also induced by non-inflammatory mechanisms, even when the activity of pro-inflammatory cytokines is not important, is an essential consideration in the development of osteoarthritis therapy [51]. Since MMP-9 is considered to be one of the most specific targets for the development of potential inhibitors for the treatment of osteoarthritis based on a certain number of previous studies, the significant inhibitory efficiency of the mixture of *A. cepa* bulbs and *S. aromaticum* spices against MMP-9 in this study suggests that it can be a component in the management of osteoarthritis.

Nitrite oxide synthase (NOS) induce damage to cartilage by increasing the activity of MMPs and downregulation of collagen biosynthesis. In addition, IL-1β and TNF-α activate the nuclear factor kappa B (NF-kB) signaling pathway by reacting with NO to produce peroxynitrite, which promotes the expression of NOS [52]. Since NOS functions as a crucial mediator in the pathogenesis of osteoarthritis, the inhibition of NOS2 is also studied as an emerging pharmacological mechanism for potential candidates for the management of joint pathologies. The effects demonstrated by the mixture of *A. cepa* bulbs and *S. aromaticum* spices against the main pro-inflammatory mediators in this study go beyond the simple confirmation of its excellent anti-inflammatory effects and provide a certain confirmation of the hypothesis that this mixture is worthy of being counted among the potential candidate against osteoarthritis.

Oxidative stress plays an important role in osteoarthritis pathogenesis [53,54] since patients with osteoarthritis are exposed to powerful oxidative stress [55]. Antioxidants are very effective in modifying oxidative enzyme levels in osteoarthritis [56]. The early stages of osteoarthritis pathogenesis are associated with an important alteration of SOD expression [57]. In patients with osteoarthritis, an increase in MDA is generally observed, a decrease in glutathione (GSH) and a decrease in Catalase (CAT) and SOD activities [54]. Peroxidation of MDA or lipids increases in plasma and synovial fluid of osteoarthritis patients and patients with rheumatoid arthritis [54]. The results of our study have shown that MIA's intra-articular injection has caused a decrease in SOD and CAT activities, a decrease in GSH levels and an increase in the level of MDA in non-treated osteoarthritis animals. The activities of SOD and CAT, as is the GSH level, significantly increased in the groups treated with the aqueous extract of the mixture *A. cepa* bulbs and *S. aromaticum* spices. These increases were significantly more important than in the untreated osteoarthritis group. These results confirm that the aqueous extract of the mixture *A. cepa* bulbs and *S. aromaticum* spices have bioactive compounds with antioxidant ownerships. This complex with *A. cepa* bulbs and *S. aromaticum* spices protects the state of the patient by anti-hyperalgesia activity, anti-inflammatory activity, delaying platelet aggregation, improving antioxidant activity and trapping free radicals, inhibiting the expression of pro-inflammatory cytokines and other factors inflammatory parameters.
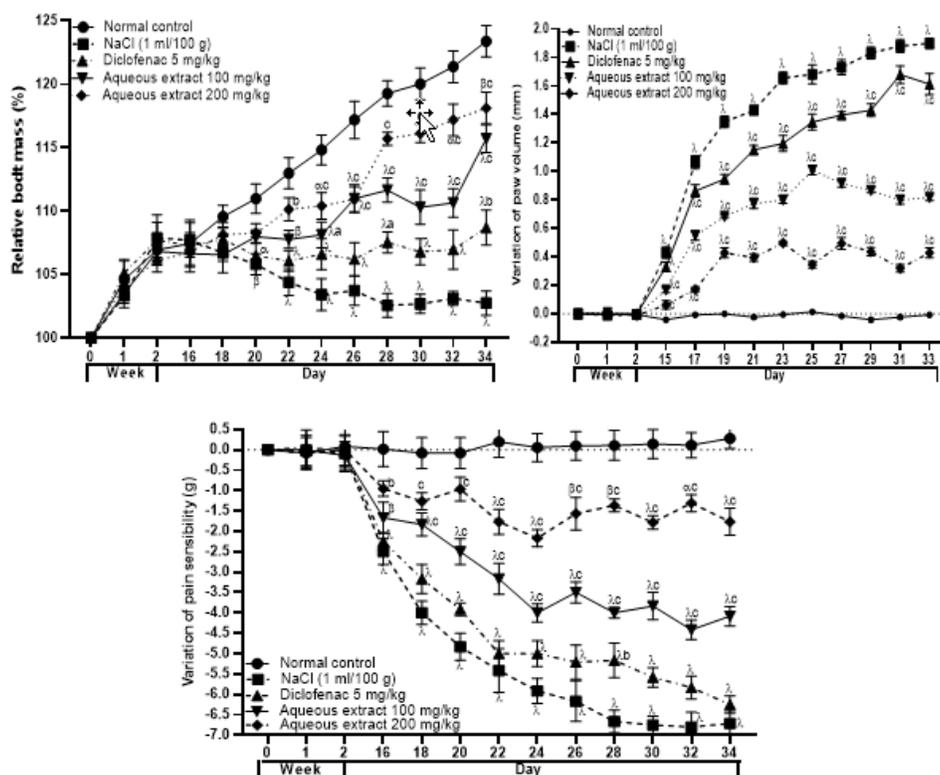

Figure 1. Effect of the aqueous extract of the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices on body mass, inflammation and hypersensitivity of the osteoarthritis induced by MIA.
Data are shown as mean ± SEM, n = 6; $^{\alpha}p<0.05$; $^{\beta}P<0.01$; $^{\lambda}p<0.001$ significant compared to the normal witness; $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant compared to the NaCl.

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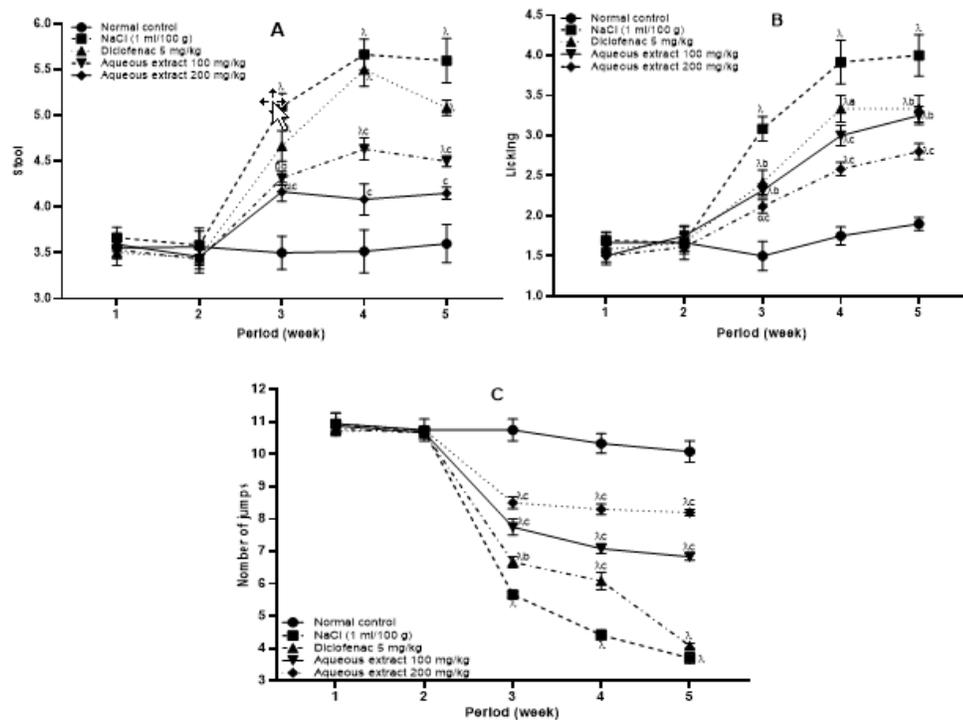
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Figure 2. Effect of the aqueous extract of the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices on the number of stools (A), the number of licking (B) and the number of jumps (C) of the rats made osteoarthritis induced by MIA.

Data are shown as mean ± SEM, n = 6; $^{\alpha}p<0.05$; $^{\beta}P<0.01$; $^{\lambda}p<0.001$ significant compared to the normal witness; $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant compared to the NaCl.

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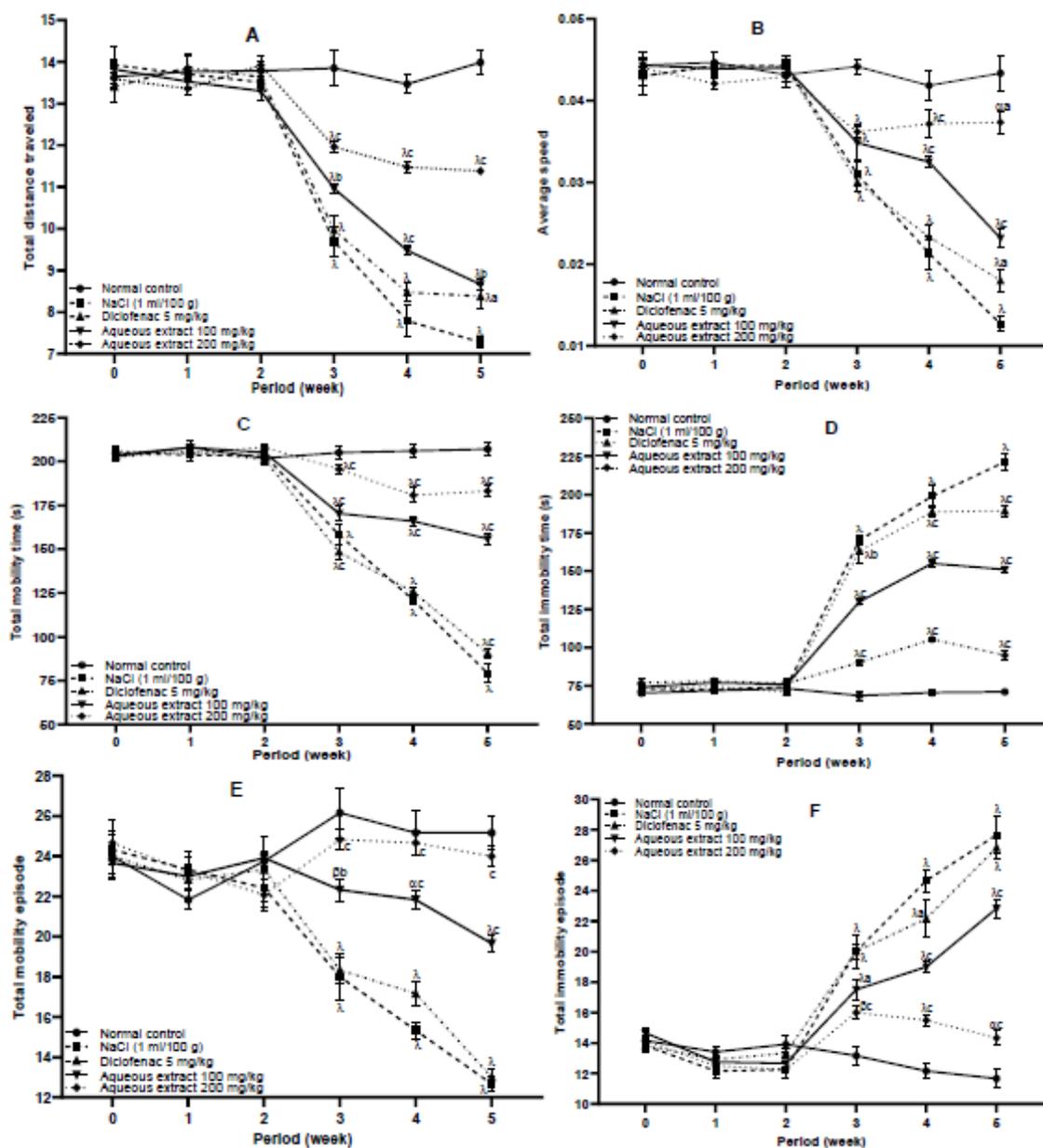
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Figure 3. Effect of the aqueous extract of the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices on the total distance traveled (A), average speed (B), total mobility time (C), total immobility time (D), mobility time episode (E) and immobility time episode (F) of the osteoarthritis induced by MIA.

Data are shown as mean ± SEM, n = 6; $^{\alpha}p<0.05$; $^{\beta}P<0.01$; $^{\wedge}p<0.001$ significant compared to the normal witness; $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant compared to the NaCl.

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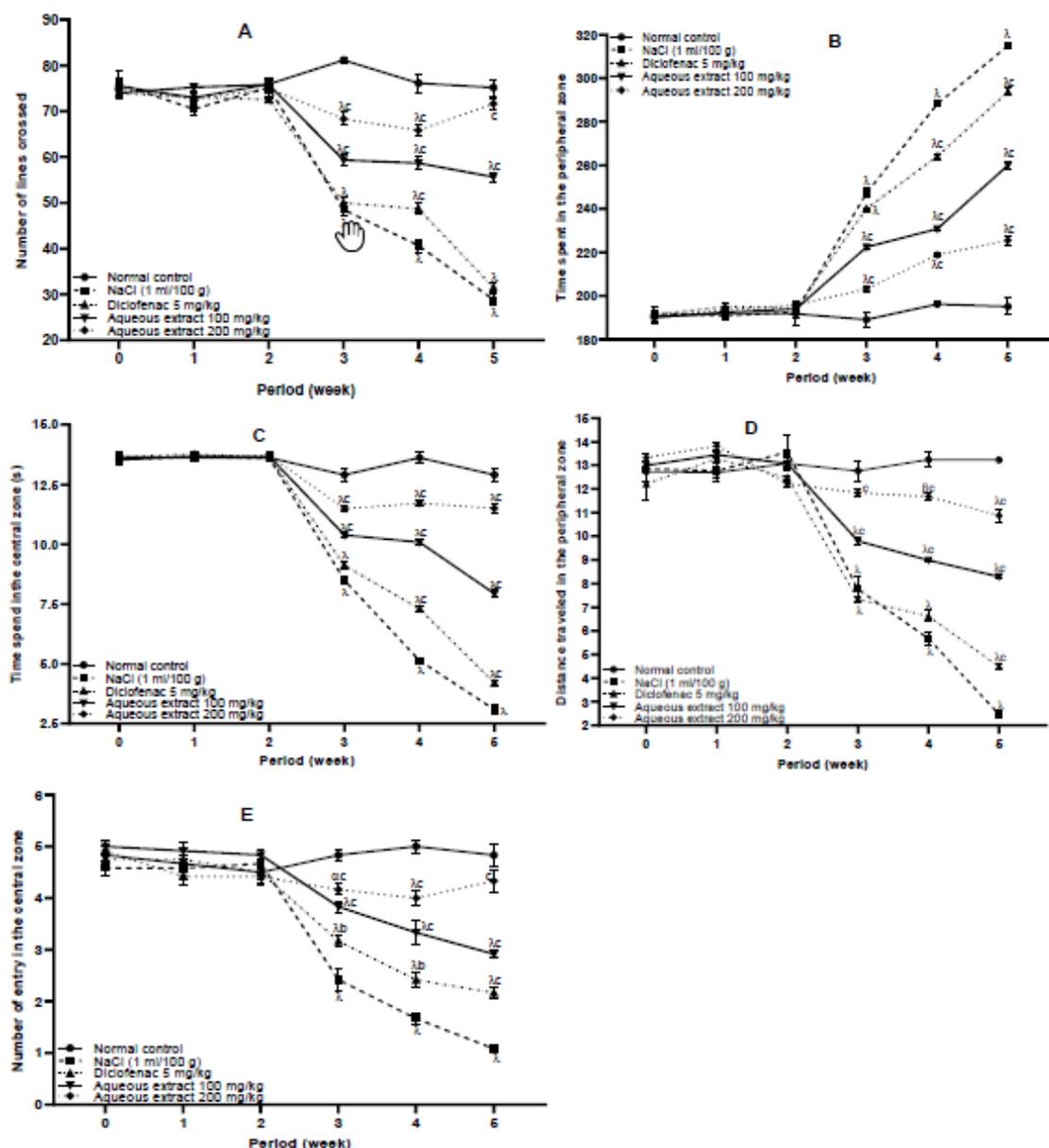
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Figure 4. Effect of the aqueous extract of the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices on the number of lines crossed (A), time spent in the peripheral zone (B), time spent in the central zone (C), distance traveled in the peripheral zone (D) and number of entry into the central zone (E) of the osteoarthritis rats induced by MIA.

Data are shown as mean ± SEM, n = 6; $^{\alpha}p<0.05$; $^{\beta}P<0.01$; $^{\lambda}p<0.001$ significant compared to the normal witness; $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant compared to the NaCl.

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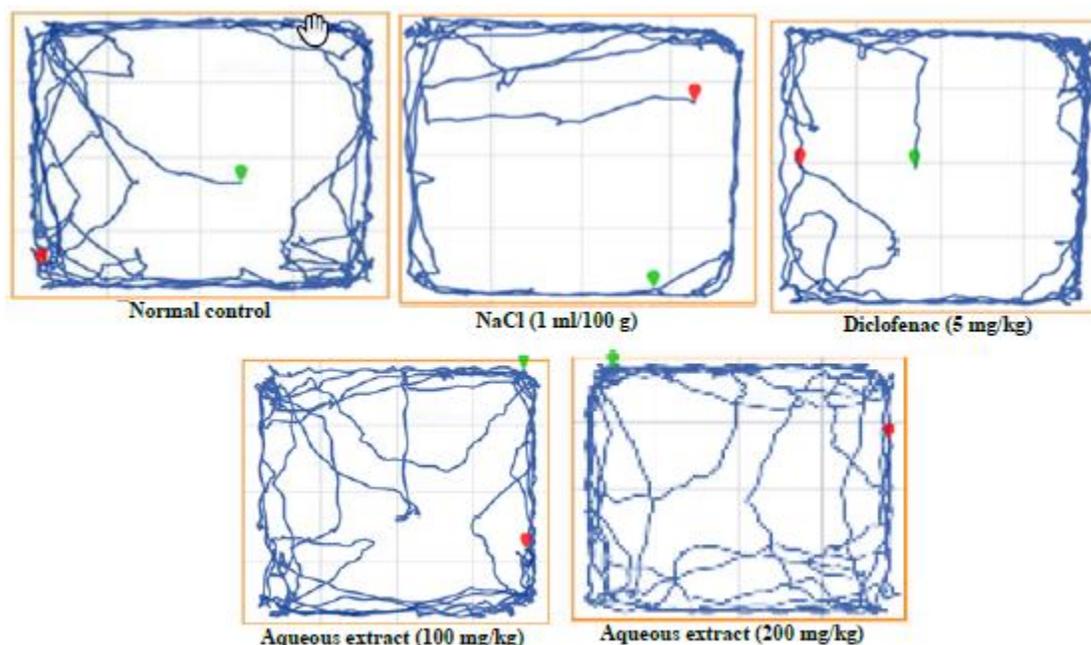
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Figure 5. Effect of the aqueous extract of the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices on spontaneous locomotion of the rats with osteoarthritis induced by MIA.

Table 1. Effect of the aqueous extract of the mixture of *Allium cepa* bulbs and *Syzygium aromaticum* spices on some parameters of the osteoarthritis rats induced by MIA.

	Dose (mg/kg)	White cell (10^3/μL)	Red cell (10^6/μL)	Hemoglobin (g/dl)	Hematocrite (%)	Platelets (10^3/μL)	MHCC (g/dl)	CHCC (pg)	Lymphocyte (10^3/μL)	Monocytes (10^3/μL)	Granulocyte (10^3/μL)
Normal control	-	11.48 ± 0.15	9.68 ± 0.29	15.12 ± 0.40	38.80 ± 1.65	459.20 ±17.43	37.50 ± 1.22	36.93 ± 0.63	4.33 ± 0.17	1.40 ± 0.04	4.95 ± 0.16
NaCl 0,9%	-	17.98 ± 0.23[ᴷ]	6.56 ± 0.31[ᴷ]	10.50 ± 0.54[ᴷ]	36.88 ± 2.92	877.00 ± 10.00[ᴷ]	27.28 ± 1.34[ᴷ]	27.35 ± 1.47[ᴷ]	8.55 ± 0.15[ᴷ]	4.98 ± 0.16[ᴷ]	7.65 ± 0.18[ᴷ]
Diclofenac	5	14.13 ± 0.44[ᴷc]	8.81 ± 0.12[c]	12.70 ± 0.77	37.71 ± 1.49	653.00 ± 18.11[ᴷc]	31.65 ± 2.39	32.93 ± 1.28[a]	6.50 ± 0.16[ᴷc]	2.32 ± 0.09[ᴷc]	6.07 ± 0.08[ac]
Aqueous extract	100	12.30 ± 0.22[ᴷc]	9.15 ± 0.11[c]	13.33 ± 0.12[b]	37.41 ± 1.15	522.13 ± 10.25[ᴷc]	31.13 ± 1.29	33.42 ± 0.77[αa]	5.01 ± 0.12[βc]	2.56 ± 0.11[ac]	5.23 ± 0.11[ac]
	200	11.55 ± 0.12[c]	9.45 ± 0.17[c]	14.02 ± 0.40[c]	38.77 ± 1.25	462.13 ± 14.27[c]	34.22 ± 1.34	35.18 ± 0.81[c]	4.49 ± 0.09[c]	1.51 ± 0.04[c]	5.01 ± 0.21[c]

Data are shown as Mean ± SEM, n = 6; ^{α}p<0.05; ^{β}P<0.01; ^{ᴷ}p<0.001 significant compared to the normal witness; ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 significant compared to the NaCl. MHCC : Mean hemoglobin corpucular content ; CHCC : Concentration hemoglobin corpucular content

Conclusion

This study shows that the mixture of *A. cepa* bulbs and *S. aromaticum* spices has a protective effect on clinical symptoms of osteoarthritis, in particular pain, functional decline and destruction of cartilage, which can be linked to its significant anti-inflammatory effects on pro-inflammatory mediators, including IL-1β, TNF-α, Consequently, we suggest that the mixture of *A. cepa* bulbs and *S. aromaticum* spices are constituents worthy of a more in-depth study to determine whether it effectively modulates the complex inflammatory pathology of osteoarthritis according to its multiple component action. However, other studies on enlarged experimental conceptions with different components and specific mechanisms will be necessary to confirm whether the mixture of *A. cepa* bulbs and *S. aromaticum* spices is indeed a promising anti osteoarthritic constituent that can enter clinical trials.

Abbreviations

IL-6 : Interleukin 6
TNF-α : Tumor necrosis factor alpha
IL-1β : Interleukin 1 beta
ROS : reactive species of oxygen
NO : nitric oxide
H_2O_2 : hydrogen peroxide
DNA : deoxyribonucleic acid
CAT : Catalase
GSH : reductase glutathione
SOD : superoxide dismutase
A. cepa : Allium cepa
S. aromaticum : Syzygium aromaticum
MIA : Mono-iodoacetate
MPO : myeloperoxidase
ALT : Alanine aminotransferase
AST : Aspartate aminotransferase
MMP-9 : matrix metaloprotein-9
NaCl : Sodium clhoride

EDTA : Ethylene diamine tetra acetic acid
MDA : Malondialdehyde
H&E : hematoxyline and eosin
ESM : standard error of mean
ANOVA : analysis of variance
NOS : Nitrite oxide synthase
NF-κB : nuclear factor kappa B
MHCC : Mean hemoglobin corpucular content
CHCC : Concentration hemoglobin corpucular content

Authors' Contribution

BJD, MM and DAB are involved in the study's conceptualization and design, data collection and analytics supervision, and manuscript revision for essential intellectual content. BJD, MM, DAB, DBM, BTFD, KEAH and YNW involved in the conceptualization and design of the study, data gathering and analysis, and manuscript drafting. BJD, MM, DAB, AAD, TYC and KMF contributed to the data analysis and interpretation, as well as to the manuscript's scientific quality. AAD, TYC and AG helped with data collection and analysis as well as manuscript review. The final document for submission was read and approved by all authors.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest

References

1. Chao Z. 2023. The Role of Inflammation in Osteoarthritis Development and Progression. *Int J Clin Rheumtol.* 18(9):295-299.
2. Glass LRD, Freitag SK. 2015. Management of orbital IgG4-related disease. *Curr Opin Ophthalmol.* 26:491-497.
3. Pan Q, Guo L, Wu J, Cai J, Liao H, Lan Q, Peng Y, He Y, Liu H. 2016. Association between IgG4 Autoantibody and Complement Abnormalities in Systemic Lupus Erythematosus. *Mediators Inflamm.* 2016:e2196986.
4. Marchev AS, Dimitrova PA, Burns AJ, Kostov R, Dinkova-Kostova A, Georgiev MI. 2017. Oxidative stress and chronic inflammation in osteoarthritis: can Nrf2 counteract these partners in crime? *Ann NY Acad Sci.* 1401:114-135.
5. De La Fuente M, Miquel J. 2009. An update of the oxidation inflammation theory of aging: The involvement of the immune system in Oxi-Inflamm-Aging. *Curr Pharm Des.* 15(26):3003-3026.
6. Axford J, Butt A, Heron C, Hammond J, Morgan J, Alavi A, Bolton J, Bland M. 2010. Prevalence of anxiety and depression in osteoarthritis: use of the Hospital Anxiety and Depression Scale as a screening tool. *Clin Rheumatol.* 29(11):1277-1283.
7. Goldring M, Berenbaum F. 2015. Emerging tartgets in osteoarthritis therapy. *Curr Opin Pharmacol.* 22: 51-63.
8. Li Y, Xiao W, Luo W. 2016. Cellular aging towards osteoarthritis. *Mech Ageing Dev.* DOI:10.1016/j.mad.2016.12.012.
9. Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J, Callahan L, Copenhaver C, Dodge C, Felson D, Gellar K, Harvey WF, Hawker G, Herzig E, Kwoh CK, Nelson AE, Samuels J, Scanzello C, White D, Wise B, Altman RD, DiRenzo D, Fontanarosa J, Giradi G, Ishimori M, Misra D, Shah AA, Shmagel AK, Thoma LM, Turgunbaev M, Turner AS, Reston J. 2020. 2019 American College of Rheumatology/Arthritis Foundation guideline for the management of osteoarthritis of the hand, hip, and knee. *Arthritis Care Res.* 72(2): 149–162.
10. Chicken B, Staines KA. 2016. New developments in osteoarthritis and cartilage biology. *Curr Opin Pharmacol.* 28: 8-13.
11. Van Spil WE, Kubassova O, Boesen M, Bay-Jensen AC, Mobasheri A. 2019. Osteoarthritis phenotypes and novel therapeutic targets. *Biochem Pharmacol.* 165: 41–48.
12. Biswajit S, Banerjee SK, De Mazumder N, Sarkar N, Alok R, Prabir M. 2019. Studies on effects of Rumalaya in osteoarthritis. *Ind Med J.* 11(74): 151-154.
13. Hema B, Gauri S, Sony S. 2022. Review: (*Allium cepa*) to study phytochemistry and health benefit. *J Pharmacogn Phytochem.* 11(4): 245-257
14. Abdelkader O, Bouchakour T. 2018. Etude de l'activité insecticide de l'huile essentielle de *Syzygium aromaticum* et *Illicium verum* vis-à-vis *Aphis spiraecola*. Mémoire de Master, Université Abdelhamid Ibn Badis-Mostaganem, Algérie, p. 68.
15. Ashwini M, Sathishkumar R. 2014. Onion (*Allium cepa*)-Ethnomedicinal and therapeutic properties. *Research Signpost.* 37(2):27-34.
16. Marrelli M, Amodeo V, Statti G, Conforti F. 2019. Biological properties and bioactive components of *allium cepa* L. Focus on potential benefits in the treatment of obesity and related comorbidities. *Molecules.* 24(1):119.
17. Kumar KP, Sampath B, Debjit, Chiranjib, Biswajit, Tiwari P. 2010. *Allium cepa*: A traditional medicinal herb and its health benefits. *J Chem Pharm Res.* 2(1):283-291.
18. Bora K, Sharma A. 2009. Phytoconstituents and therapeutic potential of *allium cepa* linn. A Review. *Phcog Rev.* 3(5):170-180.
19. Teshika JD, Zakariyyah AM, Zaynab T, Zengin G, Rengasamy KR, Pandian SK *et al.* 2019. Traditional and modern uses of onion bulb (*Allium cepa* L.): a systematic review. *Crit Rev Food Sci Nutr.* 59:S39-S70.
20. Shenoy C, Patil MB, Kumar R, Patil S. 2009. Preliminary phytochemical investigation and wound healing activity of *Allium cepa* Linn (Liliaceae). *Int J Pharm Pharm Sci.* 2(2):167-175.
21. Boukeria S, Kadi K, Kalleb R, Benbott A, Bendjedou D, Yahia A. 2016. Phytochemical and physicochemical characterization of *Allium sativum* L. and *Allium cepa* L. Essential oils. *J Mater Environ Sci.* 7(7):2362-2368.
22. Rhodes MJC, Price KR. 1996. Analytical problems in the study of flavonoid compounds in onions. *Food Chem.* 57:113-117.
23. Mlcek J, Jurikova T, Skrovankova S, Sochor J. 2016. Quercetin and its anti-allergic immune response. *Molecules.* 21(5):1-15.
24. Boukhatem F. 2017. Activité antibactérienne de l'huile essentielle de deux épices : *Syzygium aromaticum* et *Illicium verum*. Mémoire de Master, Université Abdelhamid Ibn Badis-Mostaganem, Algérie, p. 79.
25. Nathalie G. 2016. Épices et santé. Éditions Trédaniel. 24-25
26. Deli V, Nnanga Nga E, Betti JL, Etame Loe GM, Mvogo Ottou PB, Priso RJ, Foze NT, Ngo Boumsong PC, Dibong SD, Mpondo Mpondo E. 2016. Contribution des populations des villes de Yaoundé et Douala à la connaissance des plantes à tanins et à anthocyanes. *J Anim Plant Sci.* 30(3):4797-4814.
27. Norton S, Culver B, Mullenix P. 1975. Development of nocturnal behavior in albino rats. *Behav Biol.* 15(3):317–331.
28. Bădescu SV, Tătaru CP, Kobylinska L, Georgescu EL, Zahiu DM, Zăgrean AM, Zăgrean L. 2016. Effects of caffeine on locomotor activity in streptozotocin-induced diabetic rats. *J Med Life.* 9(3):275–279.
29. Zhou X, Wangb S, Ding X, Qin L, Mao Y, Chenb L, Li W, Ying C. 2017. Zeaxanthin improves diabetes-induced cognitive deficit in rats through activiting PI3K/AKT signaling pathway. *Brain Res Bull.* 132:190-198.
30. Lampropoulou-Adamidou K, Lelovas P, Karadimas EV, Liakou C, Triantafillopoulos IK, Dontas I, Papaioannou NA.. 2014. Useful animal models for the research of osteoarthritis. *Eur J Orthop Surg Traumatol.* 24(3):263-71.
31. Kawano T, Miura H, Mawatari T, Moro-Oka T, Nakanishi Y, Higaki H, Iwamoto Y. 2003. Mechanical effects of the intraarticular administration of high molecular

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weight hyaluronic acid plus phospholipid on synovial joint lubrication and prevention of articular cartilage degeneration in experimental osteoarthritis. *Arthritis Rheum.* 48(7):1923-9.

32. Salvemini D, Little JW, Doyle T, Neumann WL. 2011. Roles of reactive oxygen and nitrogen species in pain. *Free Radic Biol Med.* 51(5): 951–966.

33. Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, Sokolove J. 2016. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. *Nat Rev Rheumatol.* 12(10): 580–592.

34. Neogi T, Guermazi A, Roemer F, Nevitt MC, Scholz J, Arendt-Nielsen L, Woolf C, Niu J, Bradley LA, Quinn E, Law LF. 2016. Association of joint inflammation with pain sensitization in knee osteoarthritis: the multicenter osteoarthritis study. *Arthritis Rheumatol.* 68(3): 654–661.

35. Arendt-Nielsen L. 2017. Pain sensitisation in osteoarthritis. *Clin Exp Rheumatol.* 35 Suppl. 107(5): 68–74.

36. Leksiri S, Hasriadi, Danusi Wasana PW, Vajragupta O, Rojsitthisak P, Towiwat P. 2020. Co-administration of pregabalin and curcumin synergistically decreases pain-like behaviors in acute nociceptive pain murine models. *Molecules.* 25(18): 4172.

37. Van den Berg WB. 2011. Osteoarthritis year 2010 in review: Pathomechanisms. *Osteoarthr Cartil.* 19: 338-341.

38. David W, Maimes S. 2007. Adaptogens, Herbs for Strength, Stamina, and Stress Relief, 1st ed. Rochester Healing Arts Press, Vermont, 211-222.

39. Bârcă M, Manda G, Ciobanu AM, Bălălău C, Lupuleasa D, Burcea Dragomiroiu GTA, Pop A, Popa DE, Baconi DL. 2017. Immunomodulatory effects of methadone following methotrexate therapy in a rat model of arthritis. *Farmacia.* 65(3): 423-428.

40. Loukov D, Karampatos S, Maly MR, Bowdish DM. 2017. Monocyte activation is elevated in women with knee osteoarthritis and associated with inflammation, BMI and pain. *Osteoarthr Cartil.* 17:1063-1084.

41. Dong X, Qu S. 2022. Erigeron breviscapus (Vant.) Hand-Mazz. A Promising Natural Neuroprotective Agent for Alzheimer's Disease. *Front Pharmacol.* 13:877872.

42. Gao J, Chen G, He H, Liu C, Xiong X, Li J, Wang J. 2017. Therapeutic Effects of Breviscapine in Cardiovascular Diseases: A Review. *Front Pharmacol.* 8:289.

43. Muthu S, Korpershoek JV, Novais EJ, Tawy GF, Hollander AP, Martin I. 2023. Failure of Cartilage Regeneration: Emerging Hypotheses and Related Therapeutic Strategies. *Nat Rev Rheumatol.* 19:403–416.

44. Bi J, Zhang C, Lu C, Mo C, Zeng J, Yao M, Jia B, Liu Z, Yuan P, Xu S. 2024. Age-Related Bone Diseases: Role of Inflammaging. *J Autoimmun.* 143:103169.

45. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. 2018. Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. *Front Immunol.* 9:586.

46. Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, Jian L. 2022. Aging and Aging-Related Diseases: From Molecular Mechanisms to Interventions and Treatments. *Signal Transduct Target Ther.* 7:391.

47. Liu-Bryan R, Terkeltaub R. 2015. Emerging Regulators of the Inflammatory Process in Osteoarthritis. *Nat Rev Rheumatol.* 11:35–44.

48. Jenei-Lanzl Z, Meurer A, Zaucke F. 2019. Interleukin-1β Signaling in Osteoarthritis-Chondrocytes in Focus. *Cell Signal.* 53:212–223

49. Grillet B, Pereira RVS, Van Damme J, Abu El-Asrar A, Proost P, Opdenakker G. 2023. Matrix Metalloproteinases in Arthritis: Towards Precision Medicine. *Nat Rev Rheumatol.* 19:363–377.

50. Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, Mitchell P, Hambor J, Diekmann O, Tschesche H, Chen J, Wart HV, Poole AR. 1997. Enhanced Cleavage of Type II Collagen by Collagenases in Osteoarthritic Articular Cartilage. *J Clin Investig.* 99:1534–1545.

51. Baral P, Udit S, Chiu IM. 2019. Pain and Immunity: Implications for Host Defence. *Nat Rev Immunol.* 19:433–447.

52. Ahmad N, Ansari MY, Haqqi TM. 2020. Role of iNOS in Osteoarthritis: Pathological and Therapeutic Aspects. *J Cell Physiol.* 235:6366–6376.

53. Ostalowska A, Birkner E, Wiecha M, Kasperczyk S, Kasperczyk A, Kapolka D, Zon-Giebel A. 2006. Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. *Osteoarthr Cartil.* 14(2):139-45.

54. Surapaneni KM, Venkataramana G. 2007. Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis. *Indian J Med Sci.* 61(1):9-14.

55. Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. 2007. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. *Rheumatol Int.* 27(4):339-44.

56. Panahi Y, Rahimnia AR, Sharafi M, Alishiri G, Saburi A, Sahebkar A. 2014. Curcuminoid treatment for knee osteoarthritis: a randomized double-blind placebo-controlled trial. *Phytother Res.* 28(11):1625-31.

57. Scott JL, Gabrielides C, Davidson RK, Swingler TE, Clark IM, Wallis GA, Boot-Handford RP, Kirkwood TBL, Taylor RW, Young DA. 2010. Superoxide dismutase downregulation in osteoarthritis progression and end-stage disease. *Ann Rheum Dis.* 69(8):1502-10.