Aqueous extract of *Cucumis sativus* Linné fruit Attenuates Rheumatoid Arthritis-Associated disorders

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Abstract:

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease with presently unknown cure. However, its management involves the reduction of pain, and immunological and biochemicals disorders. Natural plants have been proven to be safe and less cost-effective treatment for arthritis management. The study aimed at evaluating the effects of the aqueous extract of *Cucumis sativus* L. fruits on inflammation and cartilage erosion in an animal model of rheumatoid arthritis. RA was induced in female wistar rats by injecting formaldehyde (2% v/v in saline solution) into the sub-plantar region of their left hind paw. Animals were then treated by daily gavage (400 mg/kg bw) of *C. sativus* L. aqueous extract or glucosamine/chondroitin (a dietary complement) for 14 days. The weight of the animals and the diameter of the edemas were recorded every 3 days. The rats were sacrificed, and the blood collected for the evaluation of hematological and biochemical parameters (C-reactive protein and plasma Ca^{2+}, Mg^{2+} levels). The aqueous extract of *C. sativus* L. exhibited an enthralling suppression in edema of treated animals at the end of the study with 64.91% of protection like glucosamine/chondroitin. In addition, it increased the levels of lymphocytes and polymorphonuclear cells and significantly reduced C-reactive protein, calcium and magnesium levels when compared to the disease control group. Thus, the current study showed that the aqueous extract of *C. sativus* L. possesses anti-arthritic activities making it therefore a promising agent for the management of disorders associated to RA.

Key words: Antiarthritic Activity, *Cucumis sativus* L. fruit, formaldehyde, inflammation.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, which affects approximately 1% of the world's population (Mcinnes et al. 2011; Guo et al. 2018). The disease happens more regularly in females than males, being mostly observed in the elderly. It is clinically manifested by joint inflammation with pain and swelling, which can lead to irreversible cartilage and bone damage (Guo et al. 2018). Its diagnosis is based on composite scales measuring the disease activity which include the Disease Activity Score (DAS-28) such as reduced joint destruction, less radiologic progression, no functional disability with addition of serology test (C-reactive protein) (Guo et al. 2018). RA is considered as a multifactorial disease, that development depend on combination of genetic and environmental risk factors as well as other risk factor such as serological alterations (Jang et al. 2022). It results from the self-activation of immune cells which leads to synovial membrane hyperplasia, infiltration of inflammatory cells (cytokines), neovascularization, cartilage erosion and destruction of mobile joints (Chunxia et al. 2011). In addition to joint involvement, RA patients often present biochemical disorders, such as, ionic homeostasis disorders including hypercalcemia and hyper-magnesia, and oxidative stress (OX) (Kerekes et al. 2014). OX is described as a condition in which the pool of reactive oxygen species (ROS) increases over time, either by their increased production, the reduction in antioxidant defenses, and/or the combination of both (Smallwood et al. 2018; Fonseca et al. 2019).

There is presently no cure for RA; the pharmacologic handling of the disease is based on the management of pain and immunological disorders with non-steroidal anti-inflammatory and immunosuppressive drugs like Methotrexate. Furthermore, biochemical disorders in RA are modulated through the intake of mineral supplements such as magnesium and calcium (Zeng et al. 2017; El-Said et al. 2022). Despite their high cost, the availability of these drugs or supplements is not always guaranteed and do not moderate the disease progression. Furthermore, they can negatively affect normal cells resulting in several toxicities (Cronstein and Aune 2020). In recent years, preclinical trials have proven that natural plant extracts and compounds such as curcumin can significantly relieve or mitigate RA (Zhao et al. 2021). In addition, a flavonoid compound quercetin was found ameliorating the synovial inflammation by inhibiting adenosine deaminase enzyme activity in arthritis rats (El-Said et al. 2022).

Cucumis sativus is a plant of the Cucurbitaceae family which is widely distributed throughout the world. It is cultivated for its fruits commonly called cucumber, which is eaten as food by

humans. *C. sativus* fruit is a rich source of bioactive compounds such as alkaloids, flavonoids, terpenoids, tannins (Siddikaet al. 2015; Uzuazokaro et al. 2019). Cucumber contains water, proteins, fats, carbohydrates, mineral matter, calcium, phosphorus, iron (1.5 mg/100 g), vitamin C (7 mg/100 g), and vitamin B (30IU/100 g) (Nema et al. 2011). In addition to its richness in these nutrients, it contains bioactive phenolic compounds (Wahid et al. 2021). Furthermore, fruits of *C. sativum* also showed reduced pain effect to the knees of moderate osteoarthritis patients in a randomized controlled clinical trial (Nash et al., 2018). However, its effect on RA management has not been proven. Thus, the current study aimed at evaluating the effects of the aqueous extract of *C. sativus* L. fruits on inflammation and cartilage erosion in an animal model of rheumatoid arthritis.

Materials and methods

1. Animals

Old female Wistar rats, aged between 10 and 12 weeks, were acquired from the Animal Physiology Laboratory of the University of Yaoundé I (Cameroon). All animal experiments were conducted in accordance with the ethical guidelines outlined in the "Guide to the Care and Use of Laboratory Animals." The practices and the related protocols were approved by the Animal Protection and Use Committee of the Institutional Ethics Committee of the Cameroonian Ministry of Scientific Research and Technological Innovation.

2. Plant material

The fruits of *C. sativus* L. were purchased in the Mfoundi Market in Yaoundé, a town in the Center Region of Cameroon. The fruits were identified in comparison to the voucher specimen N°42/NHC in the National Herbarium of Cameroon.

3. Preparation of plant aqueous extract

The whole fruits of *C. sativus* L. were weighed, washed and air dried to constant weight, blended and then macerated for 24 hours in distilled water at room temperature. Maceration was done using the proportion of 1 g of ground material per 30 mL of distilled water solvent. The supernatant was filtered with whatman paper number 3 and dried at 65°C for 72 hours in an oven. The extract obtained was stored for further use.

4. Determination of total polyphenols and flavonoids

4.1. Total polyphenols content

The total polyphenols content was quantified using the Folin-Ciocalteu (FC) reagent according to the method of Singleton and Rossi (Singleton and Rossi 1995). The plant extract (30 µL) was mixed with 1 mL of FC reagent of 0.2N concentration. The mixture was

incubated for 5 minutes at room temperature, then 0.7 mL of Na_2CO_3 solution (7%) was added. A second incubation of 30 minutes was carried out, with the tubes stored at 25°C and protected from light. The absorbance was determined at a wave length of 750 nm using a spectrophotometer. The polyphenol content was then quantified using a standard gallic acid curve.

4.2. Flavonoids content

The total flavonoid content was quantified using aluminum chloride ($AlCl_3$) according to the method of Bahorun (Bahorunet al. 1996). One millimeter of plant extract(1mg/mL) in ethanol (95%) was mixed with 1mL of $AlCl_3$ (10%). After 30 minutes of incubation, potassium acetate (1mL, 1M) was added to the mixture. The mixture was incubated at 25°C for 10 minutes. The absorbance was recorded at 420 nm using a spectrophotometer. The flavonoid content was calculated from a standard quercetin curve.

5. Evaluation of the anti-inflammatory potential of extract

The anti-inflammatory capacity of *C. sativus* was evaluated *in vitro* through its protective effect against pro-inflammatory substances on lipid and erythrocytes membranes using lipoperoxidation and hemolysis inhibition assays.

5.1. Anti-hemolytic activity

This test is based on the reaction of red blood cells when subjected to an oxidative type of attack under strictly controlled and standardized conditions (Arbos et al. 2008). Intra- and extra cellular antioxidant defense come into play to resist this aggression and maintain membrane integrity and cell functions until the membrane is modified to the point of letting out the cell content. When the endogenous erythrocytes antioxidants are consumed, the hemoglobin then gets into the supernatant. This hemolysisis monitored by spectrophotometry at 540 nm.

From the blood of a rat collected in an EDTA-tube, red blood cells were isolated from the supernatant (plasma and white blood cell layer) by centrifugation at 1500 g for 15 minutes. They were then washed 3 times with physiological saline (0.9% NaCl) and the resulting red blood cells suspension(33%hematocrit) was stirred gently and kept cool at 4°C. A volume of 0.2 mL of extract (0.5-1.75-3.25-4.75 and 6.25 mg / mL) prepared in water was mixed with 1 mL of NaCl (0.9%). The ascorbic acid used as a reference molecule was prepared under the same conditions. To the mixture was added 0.1 mL of the suspension of red blood cells. The mixture was incubated at 25°C for 30 minutes, then 0.1 mL of CuSO4 (0.1 M) was added to induce hemolysis. After 30 minutes of incubation at 25°C, the solution in each tube was

diluted 1/12 and the absorbance was read at 532 nm against a blank using a spectrophotometer.

The results were expressed as the rate of protection from hemolysis, according to the formula:

Protection rate (%)=100 x ($OD_{total\ hemolysis}$- OD_{test})/ $OD_{total\ hemolysis}$

5.2. Anti-lipoperoxidative activity

At physiological pH, $FeSO_4$ attacks polyunsaturated fatty acids, thus triggering lipid peroxidation which can be followed by an increase in the level of MDA, which absorbs at 532 nm. Antioxidants inhibit the effect of $FeSO_4$, consequently reducing the level of MDA produced (Kumar et al. 2000).

A volume of 300 µL of extract (0.5-1.75-3.25-4.75 and 6.25 mg / mL) was added to 500 µL of liver homogenate. The ascorbic acid used as a reference molecule was prepared under the same conditions. A section of rat liver was ground in NaCl solution (0.9 %). After drying, the ground material was homogenized in phosphate buffer (10% w/v). To the mixture, 100 µL of NaCl were added and lipid peroxidation was initiated by adding 100 µL of iron sulfate (15mM). The mixture was incubated at 37°C for 30 min. One milliliter (1 mL) of TBA (1%) / HCl (10%) (1/1, v/v) were then added to the solution, followed by the addition of 1 mL of ascorbic acid (6 mM). The final mixture was heated at 80°C for 20 min in a water bath, cooled and then centrifuged at 1500 g for 10 min. The optical density was read at 532 nm. The control was performed in the absence of the extract (Cheriti et al. 2016). The results were expressed as percentage of inhibition of lipoperoxidation according to the formula:

Inhibition of lipoperoxidation (%) = 100 x ($OD_{control}$ - OD_{test}) / $OD_{control}$

Where OD=Optical Density

6. Evaluation of antiarthritic activities

6.1. Arthritis induction and experimental design

To induce RA in rats, 0.4 mL of formaldehyde (2% v/v in normal saline) solution was injected into the sub-plantar region of the left hind paw of the rat (Mohanty et al. 2020). The same procedure was repeated on the third day of the experiment, to optimize the onset of RA. Then, the rats were divided into four groups (n = 5 in each group) as follows:

- Control group: non-arthritic rats which received distilled water;
- Disease control group: arthritic rats which received distilled water;
- *C. sativus* L. (400 mg/kg): arthritic rats which received 400 mg/kg of aqueous extract of C. sativus;

- Glucosamine/chondroitin: arthritic rats which received 400 mg/kg of glucosamine/chondroitin (anti- inflammatory reference drug).

The animals were orally treated with the extract and the standard drug one hour before injection of formaldehyde, and then daily for 14 days. The dose of *C. sativum* L. extract (400 mg/kg body weight) was chosen based on previous studies (Wahid et al. 2021 and Siddika et al. 2015). Reference drug was chosen according to previous clinical trials (Nash et al. 2018). Parameters including the body weight and diameter of the edema were assessed every 3 days until the end of the treatment. The percentage of variation of body weight was calculated as follows:

$$\%var = \frac{Pti - Pt0}{Pt0} \times 100$$

Where Pt_i=Weight at different times and Pt_0=Weight at initial time. The percentage of protection of paw edema was calculated using the following formula:

$$= \left[\frac{PE_{control} - PE_{treated}}{PE_{control}} \right] \times 100$$

Where PE control and PE treated are the diameters of the paw edema of the disease control and treated animals, respectively. At the end of the treatment phase, the animals were left for a fasting period of 12 hours, after which they were sacrificed by cervical decapitation under light formalin anesthesia. Blood was collected for hematological and biochemical parameters analysis.

6.2. Biochemical analysis

Plasma calcium and magnesium levels as well as C-reactive protein levels were assessed following the kit protocol.

7. Statistical analysis

Statistical analysis was done using the Statistical Package for Social Science (SPSS) software, version 10.0 for Windows. One-way Analysis of variance (ANOVA) between groups was done with LSD (Least Significant Difference). Post hoc test to compare values 2 by 2. Significant differences were detected at a 95% confidence interval. The results obtained were expressed as mean ±SD from three distinct observations.

Results

1. Phenolic content of the aqueous fruits extract of *C. sativus* L.

The levels of phenolic compounds of *C. sativus* L. fruits extract is shown in table 1. Total phenolic content was 108.22 ± 9.43 mg GAE/g of dry weight and total flavonoids content was 9.4 ± 0.5 mg QEq/g of dry powder dry

Table 1: Phenolic compounds content of the aqueous fruits extract of *C. sativus* L.

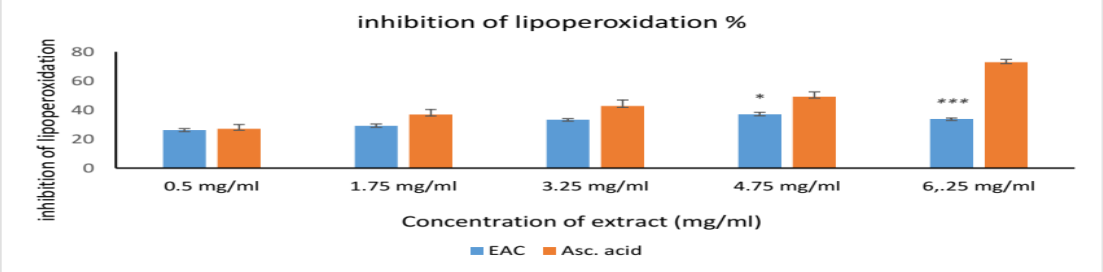
	Polyphenols (µg of Gallic acid equivalent/mg of Dry Matter)	Flavonoids (µg of Catechin Equivalent/mg of Dry Matter)
Aqueous extract of *C. sativus*	108.22 ± 9.43	9.4 ± 0.5

2. *In vitro* Anti-inflammatory effects of aqueous fruits extract of *C. sativus* L.

2.1. Effect on lipoperoxidation

Figure 1 below shows the effects of the aqueous fruits extract of *C. sativus* L. on the inhibition of lipoperoxidation, represented as inhibition percentage. The activity was measured at the different concentrations of the extract. The inhibition percentages were ranged from 26.09 (at 0.5 mg/mL concentration) to 37.03% (4.75 mg/mLconcentration) but were lower than those of ascorbic acid at the same concentrations.

Figure1: Percentage of inhibition of the aqueous fruits extract of *C. sativus* L. on lipoperoxidation



EAC: Aqueous extract of C. sativus; Asc. acid: Ascorbic acid; Values are expressed as mean ±standard error; *p < 0.05 and ***p < 0,001 in comparison with ascorbic acid value at the same concentration.

2.2. Effect of aqueous fruits extract of C. sativus L. *on hemolysis*

Figure 2 below shows the effects of aqueous fruits extract on erythrocyte attack, represented as inhibition percentage of hemolysis. The activity was recorded at the different concentrations of the extract. The inhibition percentages were inversely proportional to the dose of extract and ranged from 2.81% (6.25 mg/mL) to 45.08% (0.5 mg/mL). In comparison, the activities of ascorbic acid were dose-dependent and were higher than those of the extract.

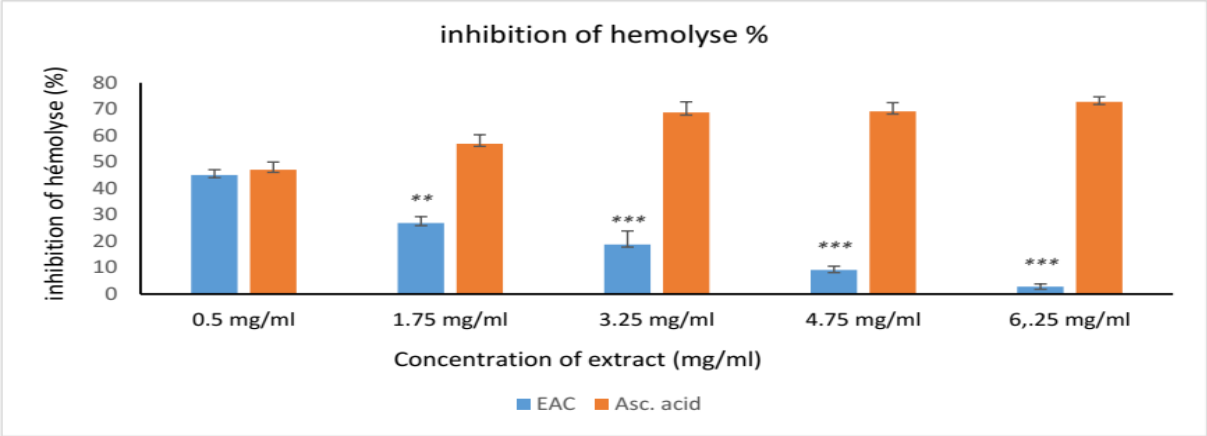


Figure 2: Percentage of inhibition of the aqueous fruits extract of *C. sativus* L. on hemolysis
EAC: Aqueous extract of C. sativus; Asc. acid: Ascorbic acid; Values are expressed as mean ±standard error; *p < 0.05 and ***p < 0,001 in comparison with ascorbic acid value at the sameconcentration.

3. Effect of the aqueous fruits extract of *C. sativus* L. on some physiological disorders associated with arthritis

3.1. Effect of aqueous extract of C. sativus L. *on body weight changes*

The change in the body weight of rats is shown in table 2. In comparison to normal control group, disease control group revealed significant increase in body weight. The administration of *C. sativus* L. extract (400 mg/kg) reduced significantly the weight gain compared to the disease control group. No significant increase in body weight were observed between *C. sativus* L. extract (400 mg/kg) and Glucosamine/chondroitin (400mg/kg) groups.

Table 2: Effect of *C. sativus* L. aqueous fruits extract on body weight changes in formaldehyde induced arthritis.

Groups	Day 0	Day 3	Day 6	Day 9	Day 14
Normal control	258±33.78 (0%)[a]	278.33±33.12 (4.85%)[a]	261.67±33.86 (1.43%)[a]	261.67±34,24 (1.42%)[a]	261±33.78 (1.18%)[a]
Disease control	217.4±28,55 (0%)[a]	233.6±31.08 (7.45%)[b]	237.4±31.03 (6.90%)[b]	227.4 ± 31.48 (4.60%)[b]	232.8±32.73 (7.08%)[b]
C. sativus (400 mg/kg)	224±9.19 (0%)[a]	233±12.14 (3.98%)[a]	234.2±9.91 (4.55%)[ed]	226.8±10.21 (1.24%)[a]	227±9.54 (1.34%)[a]
Glucosamine /chondroitine(400mg/kg)	235.5±2.61 (0%)[a]	236.5±23,95 (0.45%)[c]	237.25±24.36 (0.76%)[a]	237.5±24.56 (0.86%)[a]	239.5±24.03 (1.73%)[a]

Data are expressed as mean ± SEM, where n = 5, The values assigned with in each column to different letters are significantly different at p <0.05. The values in parentheses represent percentage of variation of body weight

3.2. Effect of aqueous extract of C. sativus L. *on the evolution of edema*

The effect of aqueous fruits extract of *C. sativus* L. on the evolution of edema after formaldehyde injection is shown in table 3. Disease control group showed a continuous increase in the diameter of edema with values ranging from 3.8 to 4.56 mm. Administration of *C. sativus* L. fruits extract at 400 mg/kg reduced significantly edema induced by formaldehyde compared to the disease control group. *C. sativus* L. (400 mg/kg) exhibited an enthralling suppression in edema of treated animals at the end of the 64.91% protection from edema. The values of percentage of protection of *C. sativus* L. were statistically similar to the values of Glucosamine/chondroitin.

Table 3: Effect of *C. sativus* L. aqueous fruits extract on evolution of edema after injection of formaldehyde into the left hind paw of rats

Groups	Day 0	Day 3	Day 6	Day 9	Day 12	Day 14
Normal control	0	0	0	0	0	0
Disease control	0	3.8±0.75 [a]	3.86±0.91[a]	4.2±0.91 [a]	4.22±1.70 [a]	4.56±1.23 [a]

C. sativus (400 mg/kg)	0	3.4±0.55 [a]	2.6±0.82 [b] (32.64%)	2.3±0.76 [b] (45.23%)	1.9±1.34 [b] (54.97%)	1.6±0.55 [b] (64.91%)
Glucosamine/ chondroitine(400 mg/kg)	0	3.75±0.5 [a]	2.62±0.75 [b] (32.12%)[b]	2.42±0.85 [b] (42.38%)	2.1±0.12 [b] (50.23%)	1.55±0.7 [b] (66.00%)

Data are expressed as mean ± SEM, where n = 5, The values assigned with different letters in each column are significantly different at p <0.05. The values in parentheses represent percentage inhibition of paw edema compared to the disease control group

3.3. Effects of aqueous extract of C. sativus on some biochemical parameters

3.3.1. Effects on plasma calcium and magnesium levels

The effects of extract on plasma calcium and magnesium levels are shown in Table 4. Plasma calcium and magnesium levels were significantly higher in disease control group (27.26 ± 2 and 4.64 ± 0.55 mg/dL) compared to normal control (20.40±1.9 and 3.7±0.15 mg/dL). Administration of *C. sativus* L. fruits extract at 400 mg/kg reduced significantly (p <0.05) plasma calcium and magnesium levels compared to disease control group. Furthermore, no significant difference was observed between the group treated with aqueous fruits extract of *C. sativus* L. and the reference group.

Table 4: Effect of *C. sativus* L. aqueous fruits extract on plasma calcium and magnesium levels

Groups	Calcium (mg/dL)	Magnesium (mg/dL)
Normal control	20.40 ± 1.9^{ac}	3.7 ± 0.15^{a}
Disease control	27.26 ± 2^{b}	4.64 ± 0.55^{b}
C. sativus (400 mg/kg)	17.34 ± 1.95^{a}	3 ± 0.35^{cd}
Glucosamine/ chondroitine (400mg/kg)	23.4 ± 2.42^{c}	2.5 ± 0.3^{d}

Data are expressed as mean ± SEM, where n = 5, the values assigned with different letters in each column are significantly different at p <0.05.

3.3.2. Effect on C-reactive protein levels

The effect of the extract of *C. sativus* on C-reactive protein was shown in table 5. A higher agglutination was observed in the disease control group which means a higher level of C-reactive protein compared to the normal control. Administration of *C. sativus* L. aqueous fruits extract lowered C-reactive protein levels.

Table 5: Effect of aqueous fruits extract of *C. sativus* L. on C-reactive protein level

Groups	Agglutination of C-reactive protein
Normal control	NA
Disease control	A++++
C. sativus (400 mg/kg)	A
Glucosamine/ chondroitine (400mg/kg)	NA

NA : No Agglutination, A: Agglutination

4. Effect of aqueous fruits extract of *C. sativus* L. on hematological parameters

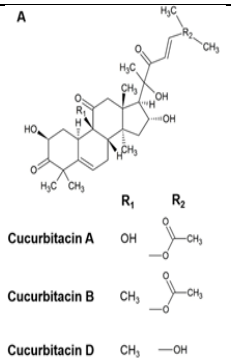
The effects of *C. sativus* L. on hematological parameters are illustrated in table 6. RBC, WBC were found to be lowered in the disease control group, but not significantly compared to the normal control group. In the group treated with the aqueous extract of *C. sativus*, the levels of WBC (14.7x103 against 4.6x103 / µL), of neutrophils (4 against 2%) and lymphocytes (7.7 against 2.2%) were significantly higher compared to the disease control group. No significant difference was observed between the reference drug and the group treated with the extract on all the hematological parameters.

Table 6: Effect of aqueous fruits extract of *C. sativus* L. on blood cellular components

Parameters	Normal control	Disease control	*C. sativus* (400 mg/kg)	Glucosamine/ chondroitin (400mg/kg)
WBC ($x10^3$ μL^{-1})	8.05 ± 6.32^{ac}	$4.6\pm2,54^{a}$	$14,7\pm4.0^{b}$	13.53 ± 4.25^{cb}
RBC ($x10^6 \mu L^{-1}$)	6.83 ± 1.71^{a}	5.8 ± 2.00^{a}	6.6 ± 1.35^{a}	6.93 ± 0.90^{a}
Hemoglobin (g/dL)	11.67 ± 3.30^{a}	9.80 ± 2.00^{a}	11.1 ± 2.13^{a}	12.1 ± 2.0^{a}
Hematocrit (%)	44.8 ± 13.10^{ab}	36.60 ± 11.40^{a}	42.4 ± 7.35^{ab}	46.82 ± 9.01^{b}
MCV(fL)	65.23 ± 3.75^{a}	$60.06\pm3,6^{a}$	65.23 ± 4.5^{a}	$67.30\pm7,53^{a}$
MCH (pg)	17.00 ± 0.72^{a}	18.00 ± 4.00^{a}	17.0 ± 2.0^{a}	$17.42\pm0,93^{a}$
MCHC(g/dL)	23.03 ± 0.72^{a}	$28.00\pm4,70^{a}$	26.0 ± 1.14^{a}	$26.00\pm1,39^{a}$
Platelets ($x10^3$ μL^{-1})	472.67 ± 206.82^{a}	$423.60\pm349,3^{a}$	503.00 ± 97.03^{a}	472.00 ± 174.19^{a}
Neutrophils (%)	2.70 ± 2.00^{ab}	2.00 ± 0.40^{a}	4.00 ± 2.06^{b}	2.80 ± 1.05^{ab}
Lymphocytes (%)	5.01 ± 4.3^{ab}	2.20 ± 2.04^{a}	7.7 ± 2.6^{b}	8.74 ± 2.10^{b}
Monocytes (%)	0.26 ± 0.1^{a}	0.70 ± 0.35^{ab}	1.13 ± 0.6^{b}	1.14 ± 1.00^{b}

WBC:white blood cells ;RBC:Red blood cells;MCV:Mean CorpuscularVolume; MCH:Mean Corpuscular Hemoglobin;MCHC:Mean Corpuscular Hemoglobin Concentration. Data are expressed as mean±SEM. The values assigned with different letters in each column are significantly different at $p < 0.05$.

5. Summary showing pharmacological proprieties of the major compounds from *C. sativus*

Compound names/family	Structure	Pharmacological properties	References
Cucurbitacins A, B, D		Hepatoprotective antineoplastic, antibacterial, COX 2 inhibition anti-fungal anti-inflammatory cardiovascular anticoagulant	Rajasree et al. 2016. Miro, 1995; Peters et al. 1997; Yesilada et al., 1998
Cardiac glycosides		Anti-inflammatory cardiac activity	Wahid et al. 2021
Tannins	/	Astringent	Wahid et al. 2021

metal ion chelating
proton precipitating
antioxidant

Discussion

The evaluation of the biological properties of *C. sativus* fruits extract in relation to their protective properties on some disorders associated with rheumatoid arthritis was the subject of this study. Scientific literature recognizes the beneficial effects of certain secondary metabolites such as polyphenols or flavonoids in the management of inflammation (Oliviero et al. 2018; Mohanty et al. 2020). In the current study *C. sativus* L. aqueous fruits extract showed good polyphenol and flavonoid contents. This is in line with previous studies which also found polyphenols such as tannins in *C. sativus* extracts (Nema et al. 2011). Arthritis is a chronic disease whose trauma is partly correlated with the extensive secretion of pro-inflammatory mediators that mediated oxidative stress (Suboh et al. 2004). In fact, during inflammation process, immune cells produce more reactive oxygen species (ROS) (Wood et al. 2003) which alter antioxidant defense and promote membrane lipid peroxidation, thus activating the inflammatory response (Hussain et al. 2016). Indeed, it becomes easy to admit the potential cross-talk between oxidative stress and RA, as such autoimmune disease characteristically represents an entity of chronic systemic inflammation (Agca et al. 2017). It therefore seemed logical to us to first carry out an in *vitro* study of the effects of *C. sativus* L. aqueous fruits extract on lipid peroxidation and erythrocyte hemolysis induced by pro-inflammatory mediators. Erythrocytes membrane structurally resembles the lysosomal membrane. *C. sativus* L. showed good inhibition of lipid peroxidation and erythrocytes hemolysis at low concentrations. The effects of extract could be justified by the ability of polyphenols to reduce pro-inflammatory compounds (Suboh et al. 2004). In fact, tannins content in ethanol extract of *C. sativa* were found to have metal ion chelating, proton precipitating and antioxidant properties that accelerate wound healing and decrease inflammation of membrane (Wahid et al. 2021).

For assessing anti-arthritic effects of aqueous extract of *C. sativus* L., formaldehyde-induced arthritis model has been used. This model is very similar to human arthritis and is used to screen anti-arthritic and anti-inflammatory agents (Greenwald 1991). In the present study the injection of formaldehyde into the left hind paw of the rats induced a continuous increase in the diameter of edema. In fact, formaldehyde injection causes pain and inflammation added to proteins denaturation at the injected region. Denatured protein will then trigger an immune

response which causes production of pro-inflammatory mediators that further worsening the condition (Thurston et al. 2010; Qasim et al. 2021). In contrast, administration of aqueous extract of *C. sativus* fruits L. (400 mg/kg) showed a protective effect (64 %) against formaldehyde-provoked edema. This protective effect was higher than that found with aqueous extract of *Achyranthes Aspera* (Amaranthaceae) which showed only 30 and 38.33% of paw inhibition at doses of 250 mg/kg and 500 mg/kg respectively after 21 days of formaldehyde injection in rats (Chinnasamy et al. 2019). In addition, *C. sativus* L. fruit (400 mg/kg) showed significant reduction in paw thickness until the 14th day that was not observed with *T. communis* (Amraoui et al. 2019). In fact, *T. communis* methanolic extract at dose of 150 mg/kg showed significant reduction in paw only on the 2nd day and 4th after formaldehyde injection, and did not show any significant effect at doses of 300 and 600 mg/kg. All of these suggest the strong anti-arthritic effect of *C. sativus* L. fruits aqueous extract. This in agreement with Wahid et al. (2021) who show strong anti-inflammatory effects of *C. sativus* seed extracts at different doses through significant reduction in paw edema as compared to control. In fact, it was shown that *C. sativus* is a rich source of several bioactive compounds particularly tetracyclic terpenoids type cucurbitacin. These compounds have strong anti-inflammatory activity which is principally due to the inhibition of the cyclooxygenase (COX) enzymes (Miro, 1995; Peters et al. 1997). The protective effect of the extract was comparable to that of the standard drug Glucosamine/ chondroitin.

With the progression of RA, we observed several changes in hematological and biochemical markers which acted as useful tools for estimating the antiarthritic ability of any test agent (Zhu et al. 2014). In the current study, a slight change in hematological parameters were found between the rat groups. However, an increase in C-reactive protein (CRP) was observed when compared disease control to normal control. This observation is consistent with the work of Qasima et al. (2019) who found an elevated level of CRP in arthritic rats. The rise in CRP may be due to the immune system's activity against inflammatory substances (Bihani et al., 2014). Indeed, the body counteracts the inflammatory activity through leukocyte infiltration that damages the tissue and lysosomal membranes (Kumar et al., 2011). Damaged lysosome tissue triggers the release of phospholipase A2 (PLA2) that mediates the hydrolysis of phospholipids to lysophospholipids and free fatty acids, which are considered precursors of inflammatory mediators. Meanwhile, the CRP level was decreased when rats were treated with *C. sativus* L. aqueous fruits extracts after 14 days. In fact, it has been found that *C. sativus* fruits are able to inhibit phospholipase A2 and prostaglandins synthase activities

thereby reduce inflammatory process (Uzuazokaroet al. 2019). Rheumatoid Arthritis is also associated with disorders of calcium and magnesium ions homeostasis (Zeng et al. 2017). In the present study, we found higher levels of plasma calcium in arthritis rats as compared to normal control. In fact, the erosion of cartilage, one of the pathological features of arthritis, is accompanied by the release of minerals into the blood. On the other hand, citrullination increases under arthritic conditions, given the high levels of the citrullinated peptide. This is a metabolic pathway catalyzed by a calcium-dependent enzyme (Radovanović-Dinić et al. 2018). The combination of these two mechanisms may justified the high level of calcium observed in arthritis rats. We also found increased level of plasma magnesium. This result is correlating with that found by Brenner et al. (2017). Treatment with the extract of *C. sativus* L. significantly lowered magnesium and calcium levels confirming its anti-arthritis effects. Indeed low-magnesium level reduces synovial expression of highly inflammatory cytokines-producing Th17 T cells such as interleukin-17 (IL-17) implicated in the pathogenesis of several autoimmune diseases including RA (Brenner et al. 2017). Thus, aqueous extract of *C. sativus* fruits L. (400 mg/kg) may protect against cartilage erosion and bone loss. This supports its beneficial effects in osteoarthritis patients in clinical trial study (Nash et al. 2018). Furthermore, the correlation between obesity and arthritis is well established (Medina Espinoza et al. 2014). At the end of the experimental phase, it was observed a significant increase in body weight in disease control rats, compared to normal control. The administration of aqueous extract of *C. sativus* fruits L. led to a significant reduction of body weight gain compared to disease control rats. Many authors have noted the beneficial effects of weight loss in the fight against arthritis. In fact, a decrease in overweight is accompanied by the reduction of stress on the joints, therefore promoting their repair and integrity (Hiligsmann et al. 2013).

Conclusions

The current study showed that the extract possesses both anti-inflammatory and anti-arthritic activities. Based on the results, anti-inflammatory effect includes the inhibition of edema formation. As for anti-arthritic activity, it may be due to decrease in the CRP rate, as well as a reduction in the loss of Ca^{2+} and Mg^{2+} in the joints. Due to these simultaneous beneficial effects, aqueous extract of *C. sativus* fruits L. (which is a functional food) presents itself as a good candidate for the prevention and the management of the disorders associated with rheumatoid arthritis.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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