Journal of Health Sciences

RESEARCH ARTICLE Open Access

Anti-inflammatory potential of polyphenols: Combining *in silico* prediction and *in vivo* data

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint inflammation and destruction, leading to significant pain and disability. Adenosine deaminase (ADA) is identified as a biomarker for RA's inflammatory process. This study aims to investigate the potential of flavonoids and phenolic acids to inhibit ADA activity (*in silico*) and evaluate their anti-inflammatory effects in a RA model (*in vivo*).

Methods: The molecular docking study was conducted using YASARA Structure 19.12.14. software following the Auto Dock 4.2 protocol. A rat model with pristane-induced arthritis was used to test the anti-inflammatory effect of selected polyphenols. The consistency of the development of the rat model was evaluated through the following indicators artistic score, paw volume, and body weight. Quercetin was administered intragastrically at doses of 150 and 400 mg/kg over 15 days. The C-reactive protein (CRP) level in serum was measured with an automatic biochemical analyzer. Statistical analyses were performed using SPSS 29.0.2.0.

Results: Molecular docking simulations showed flavonoids inhibited ADA activity with inhibition constants ranging from 0.012 mM to 0.190 mM. In the *in vivo* RA model, quercetin significantly reduced joint inflammation and serum CRP levels at a higher dose of 400 mg/kg.

Conclusion: Quercetin shows promise as an anti-inflammatory agent for RA by targeting ADA, suggesting that flavonoid-rich plant extracts could enhance RA treatment.

Keywords: Flavonoids; phenolic acids; rheumatoid arthritis; inflammation; C-reactive protein

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that primarily affects the joints, causing inflammation, pain, stiffness, swelling, and eventual joint deformity (1). RA commonly targets the synovial lining of joints, leading to erosion of cartilage and bone, and is connected with progressive disability, premature mortality, and socioeconomic burdens (1,2). The initial joints commonly affected include the metacarpophalangeal (MCP), proximal interphalangeal (PIP), wrists, and metatarsophalangeal joints. A characteristic deformity seen in RA is Boutonniere's deformity, characterized by swelling of the PIP and MCP joints accompanied by ligament laxity (3). The causes of RA are unknown. Risk factors include smoking, obesity, and exposure to air pollution. Women and older people have a higher risk of developing RA (4). Despite the ongoing ambiguity surrounding the etiology of RA, scientific investigations have revealed compelling evidence supporting the involvement of both hereditary predisposition and enviro (mental triggers in the pathogenesis of the disease (1). The initiation of RA stems from the combination of the patient's genetic susceptibility, which prompts the generation of autoreactive T and B cells, and an external triggering event, such as viral and bacterial infection or tissue damage, both acting as distinct and pivotal factors in diseases onset (1). Excessive activity of B and T lymphocytes, macrophages, synovial-like fibroblasts, matrix metalloproteinase release, and the production of cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α) lead to the enduring symptoms of chronic pain, stiffness, tenderness, heat, and joint swelling (4).

C-reactive protein (CRP) serves as an essential marker for tracking the progression of RA (5). It is consistently detected in the active phase of RA among nearly all individuals and exhibits a strong correlation with erythrocyte sedimentation rate (5). CRP functions as a general indicator of systemic inflammation and tends to be elevated in RA patients. Some studies have even noted a higher occurrence

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Submitted: 15 January 2023/Accepted: 28 May 2024

DOI: https://doi.org/10.17532/jhsci.2024.2481

UNIVERSITY OF SARAJEVO
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of elevated CRP levels in serum samples of RA patients before the onset of the disease (6). In the context of RA, various cytokines play a role in virtually all aspects of joint inflammation and damage. Elevated levels of pro-inflammatory cytokines contribute to synovial tissue proliferation, leading to damage in articular cartilage and the destruction of adjacent bone structures (7).

On the other side, when examining the current state of scientific knowledge related to mediators associated with the progression of RA, adenosine deaminase (ADA), also known as adenosine aminohydrolase, emerges as a notable biomarker for inflammatory processes. ADA serves as a valuable parameter for not only estimating the disease prognosis but also monitoring the therapeutic effectiveness of established anti-inflammatory treatments (6). ADA functions as an enzyme responsible for the irreversible hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine, accompanied by ammonium isolation (8). During inflammatory joint conditions like RA, the enzyme is released into the synovial fluid, leading to a significant increase in its activity. A prior study has explored the functional implications of ADA, including the development of several ADA inhibitors (8).

Over the past two decades, advancements in RA treatment have stemmed from a deeper comprehension of its pathogenic pathways and the subsequent creation of targeted pharmaceuticals. These newer agents exhibit considerable efficacy in ameliorating disease progression; however, their application is accompanied by significant adverse effects, presenting enduring therapeutic complexities and perioperative hurdles (9). These solutions include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, both synthetic disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX) and c-Jun N-terminal kinase inhibitors, as well as biologically derived DMARDs such as TNF inhibitors, IL-6 inhibitors, and B cell-depleting drugs (10). MTX stands as the recognized standard treatment for RA, largely due to its anti-inflammatory and immunosuppressive attributes (11). Combination therapy with MTX also could be effective in managing RA by targeting different aspects of the immune system (12).

MTX exhibits multiple anti-inflammatory effects that are, in part, mediated by adenosine. Notably, ADA plays a substantial role in accounting for the variations in MTX responses among individuals with RA (6). Consequently, it appears that the serum level of ADA could serve as a valuable parameter for both predicting the disease prognosis and monitoring the therapeutic outcomes of MTX (6,13-15). However, the use of MTX can have harmful effects on normal cells, leading to various toxicities. Reports also indicate that MTX's adverse effects extend to the neurological, gastrointestinal, reproductive, respiratory, urinary, cardiovascular, and immune systems (10,16). Despite extensive research and the introduction of new therapeutic methods and medications, significant unwanted side effects remain associated with these treatments, and a substantial number of patients do not respond favorably to existing therapeutic strategies. Continued research is being conducted to develop an effective natural anti-inflammatory agent for RA, free of side effects.

Flavonoids and phenolic acids, which are natural polyphenolic compounds, have demonstrated remarkable potential as agents for modifying inflammation in various RA models, owing to their antioxidative and anti-inflammatory properties (5,17). Flavonoids include a group of compounds formed by connecting two phenyl rings with phenolic hydroxyl groups through the central three carbon atoms (17). This diverse group of compounds consists of various subclasses that are differentiated by differences in their chemical structure, primarily associated with factors such as the quantity and position of methyl and hydroxyl groups, the type of glycoside linkage, the number and size of carbohydrate molecules, and the presence of sulfone groups (18). Based on differences in their structural characteristics, particularly the oxidation state of the C ring, flavonoids can be categorized into seven distinct subclasses: Flavonols, flavones, isoflavones, anthocyanins, flavanones, flavanols, and proanthocyanidins (19). The chemical diversity within this extensive group of polyphenols contributes to their wide range of biological properties (20).

Flavonoids display a wide range of biological activities, including antioxidant, anti-inflammatory, immunomodulatory, cardioprotective, antimicrobial, antiviral, antibacterial, antiparasitic, antifungal, and anticancer properties (17).

Phenolic or phenol carboxylic acids stand as a prominent category among plant phenolic compounds. Phenolic acids can be primarily classified into two distinct sub-groups: Hydroxybenzoic and hydroxycinnamic acids (21). In recent years, phenolic acids have gained attention due to their biological and pharmacological attributes, revealing potential health benefits. Their recognized characteristics encompass anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic properties (22). Flavonoids exhibit well-documented antioxidant activity, alongside potent anti-inflammatory capabilities by modulating the activity of key pro-inflammatory mediators such as IL-1ß, IL-2, IL-6, interferon-gamma, TNF-α, and chemokines across diverse cell types (23,24). They also manifest pro-apoptotic effects via caspase activation, impede cartilage and bone degradation, and regulate angiogenesis (24).

Recognizing the anti-inflammatory potential of polyphenols, as well as the advantages of less toxic side effects, this study aims to investigate the difference in the binding affinity of two classes of polyphenols: flavonoids and phenolic acids to the ADA enzyme *in silico*. Furthermore, the *in vivo* study was conducted on a pristane-induced model of RA to confirm the anti-inflammatory potential of *in silico-selected* polyphenols.

METHODS

The molecular docking study was performed using the software YASARA Structure 23.9.29 (25,26) following the AutoDock 4.2 protocol (27). The crystal structure of murine ADA enzyme (PDB ID: 3KM8) was downloaded from RCSB Protein Data Bank (https://www.rcsb.org) and prepared for docking analysis by removing water molecules, adding polar hydrogen atoms, and optimized in the AMBER03 force field (28). For the ligands, the energies were minimized, and geometries were optimized on the 3D structures using the MM2 force field (29,30) in Chem3D Ultra 16.0.1.4. software. The molecular docking was conducted in the area defined by setting up the cuboid

search box 5 Å larger than a specific binding pocket where 9-deazainosine used to be complexed within the enzyme. The Lamarckian genetic algorithm was employed with the following parameters: 20 docking runs with a maximum of 15,000,000 energy evaluations and 27,000 generations for each run, with a grid point spacing of 0.375 Å, providing this way the lowest energy (kcal/mol) and dissociation constants (mM) for docked poses. YASARA Structure was also used for the visualization of the ligand-enzyme complexes and image production. Quercetin (3,3,′4′,5,6-pentahydroxyflavone, 95% HPLC, Sigma Aldrich), Tween 80 (Sigma Aldrich), Sodium chloride 0.9% physiological saline (Vioser S.A.), Pristane (2,6,10,14-tetramethylpentadecane, C19H40, Thermo Scientific), ketamine (100 mg/mL, Kataminol, MSD Animal Health-Merck and Co) and xylazine (2%, Xylazine Bio, Bioveta).

A total of twenty healthy male and female Wistar rats, with an average weight of 250 g, were acquired from the University of Sarajevo's Faculty of Veterinary Medicine in Sarajevo, Bosnia and Herzegovina. maintained at 24 ± 2°C, humidity 55 ± 5% on a 12:12 h light-dark cycle throughout the study. These rats were randomly assigned to group housing, with 3-4 rats per cage, ensuring that female and male rats were separated to prevent any potential mating interactions. During the experiment, rats housed in cages were monitored and evaluated by trained veterinary professionals, including a veterinarian and veterinary technician from the University of Sarajevo-The animals were acclimatized for a week to adjust to laboratory surroundings before the experiments. They were kept on standard laboratory chow with tap water *ad libitum* and the husbandry room was Faculty of Veterinary. Animals' health and welfare were assessed throughout the study, observing parameters such as behavior, physical condition, and cage environment, according to established protocols for animal care and experimentation. The housing conditions and experimental procedures adhered to the approved protocols of the Ethics Committees at both the University of Sarajevo's Faculty of Veterinary Medicine (Number: 07-03-325-2/22) and Faculty of Pharmacy (Number: 0101-3104/22). Efforts were made to minimize animals' suffering and to reduce the number of animals used.

Wistar rats were anesthetized before the induction procedure using a mixture of ketamine (100 mg/mL) and xylazine (2%). The rats were positioned in a prone stance, and intradermal injection of 150 μL of synthetic pristane was administered on the dorsal side of the tail base on Day 0 (31). The pristane-induced arthritis (PIA) model in rats was evaluated using arthritis score, paw volume, rat body weight, and serum CRP levels.

Rat weights were recorded at least twice a week starting from the initial scoring day. The paws were inspected visually every 2 weeks, with a maximum of 15 points possible per paw. Each inflamed knuckle or PIP joint accounted for one point, while the ankle received a score from 1 to 5 based on the level of inflammation. Deformities without erythema were not scored. The assignment of arthritic scores was conducted in accordance with the Guidelines for PIA (31). Paw volume was measured using a caliper, and the unit of measurement used was mm (millimeters), allowing for precise quantification of inflammatory swelling in the rat paw. To measure inflammation in the paw, daily hind paw caliper measurements were in the metatarsal taken for each rat. Due to heterogeneities in disease induction, the most inflamed paw from each animal based on caliper scores was enrolled weekly. The initial weighing was conducted on a technical scale before the induction of RA to establish baseline weight. Following RA induction, each rat was weighed once a week. The unit of measurement for weight was grams (g).

When each rat reached its maximum arthritis score, blood samples were taken from the tail to prepare serum. Rat blood samples were subjected to centrifugation by collecting blood into tubes with yellow caps (gel + clot activator) at room temperature for 30 minutes to allow the coagulation process. Subsequently, centrifugation was carried out at 2000 × g for 15 minutes at room temperature. The supernatant (serum) was then collected using a micropipette and transferred into sterile vials suitable for placement in the biochemical analyzer. The automatic biochemical analyzer was used to measure the serum CRP levels. The quantification of serum CRP values is expressed in the measurement unit mg/dL.

In the administration phase, the rats were divided into four groups: the control group, the model group, the low quercetin administration (150 mg/kg) group, and the high quercetin administration (400 mg/kg) group. The administration group received quercetin, either at doses of 150 mg/kg or 400 mg/kg, which is suspended in a 5% Tween 80 in sodium chloride 0.9% physiological saline. The treatment was administered as a single dose for 15 days. After the 15-day treatment period, blood samples were taken from each rat's tail. The same procedure was conducted during the separation of serum from the whole blood of the rats. Serum CRP level was quantified using an automatic biochemical analyzer. The quantification of serum CRP values is expressed in the measurement unit mg/dL.

SPSS 29.0.2.0 software (SPSS, Inc., Chicago, IL, USA) was used for the statistical analyses of the data. Data were presented as mean ± SD. A group comparison of non-normally distributed data were preformed using Kruskal–Wallis. Post hoc significance values were adjusted by the Bonferroni correction for multiple tests. All P-values represent bilateral probability, and the level of significance α is 0.05.

RESULTS

Docking results were assessed based on the binding energy and dissociation constant between the flavonoids, phenolic acids, and ADA enzymes. Results of molecular docking indicated that flavonoids have lower binding energy (kcal/mol) compared to phenolic acids toward ADA enzyme, with significantly lower dissociation constant [mM]. Binding energy values ranged from −10.82 to −9.18 kcal/mol for flavonoids, while the values for the phenolic acids ranged from −7.95 to −7.40 (kcal/mol). Dissociation constants for flavonoids ranged from 0.012 to 0.190 mM, while phenolic acids had dissociation constants ranging from 1.50 to 3.79 mM. The docking results were ranked based on the binding energies and dissociation constants of flavonoids/phenolic acids-enzyme complexes, as shown in Table 1.

TABLE 1. Summarized docking results of polyphenols with ADA enzyme

Active compound	Binding energy (kcal/mol)	Dissociation constant (mM)
Myricetin	−10.82	0.012
Luteolin	−10.10	0.040
Isorhamnetin	−9.99	0.048
Quercetin	−9.76	0.071
Apigenin	−9.59	0.094
Kaempferol	−9.18	0.190
Caffeic acid	−7.95	1.50
Neochlorogenic acid	−7.86	1.74
Chlorogenic acid	−7.40	3.79

All the flavonoids seemed to form hydrogen bonds between their 7-hydroxy group and the Leu 62 residue of the enzyme, as shown in Figure 1. (Yellow-dotted lines). In addition, myricetin formed an H-bond between its 2-phenyl substituent's 3'-hydroxy group and Gly 184 residue (Figure 1A). It is interesting to note that all of the tested flavonoids are bound to the enzyme similarly, with the 2-phenyl substituent directed toward the zinc atom. However, no apparent interaction was observed between zinc's atom and the ligands' groups.

Out of 20 rats, 100% developed RA after pristane administration. After receiving pristane injection, the rats developed RA with onset occurring in the 2nd week. Onset and maximum arthritis scores occurred on days 9.5 ± 2.12 and 28.5 ± 3.54, respectively. On the 8th day of disease induction, female rats were assigned arthritic scores, while in male rats, the first arthritic score was assigned on the 11th day (Figure 2). The maximum arthritis score in the PIA rat model was achieved on day 31, assigning 60 points to each rat (Figure 2). The evaluation of arthritis score, paw volume, body weight, and serum CRP levels confirmed consistent PIA in rats.

Rats that were immunized with pristane exhibited normal weight gain before the clinical disease onset. The severity of arthritis was proportional to the decline in body weight, indicating it as a marker of disease activity (Figure 3). Body weight remained unchanged in the control group. The disease symptoms typically began and persisted in the hind paw ankles, which became swollen as it progressed (Figure 4).

During the whole course of treatment, serum CRP levels in the model group were significantly higher than those in the control group ($p < 0.05$). The serum CRP value of rats in the low quercetin administration group and high quercetin administration group gradually decreased. There was no significant difference in serum CRP value between the model and the low quercetin administration group ($r = 0.127$, $p > 0.05$). On the end of the experiment, the serum CRP value of the rats in the high-administration group was lower than that of the rats in the model group ($p < 0.05$), and the serum CRP value of the rats in the high-administration group was lower than that of the rats in the control group ($p < 0.05$).

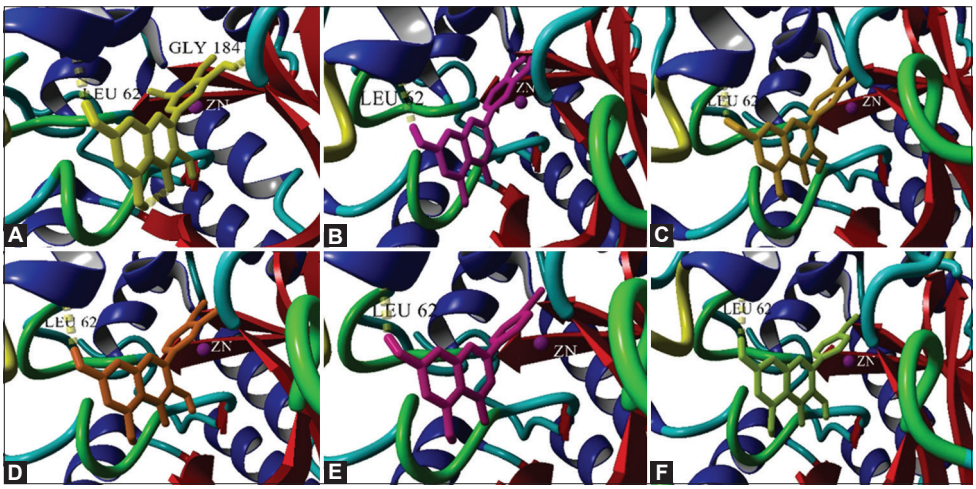
DISCUSSION

The molecular docking technique utilized in this study predicts the molecular interactions between polyphenols, which are potential anti-inflammatory agents, and the targeted enzyme ADA. ADA catalyzes the conversion of adenosine to inosine, contributing to the regulation of purine nucleoside levels (8). The ADA is particularly relevant in the context of inflammatory and immune responses, as adenosine acts as a potent anti-inflammatory molecule (32-34). The analysis of molecular docking outputs, such as binding affinity and dissociation constant, was applied to predict the potential ligands among analyzed polyphenols fo*r in vivo* study purposes, providing insights into their inhibitory or modulatory anti-inflammatory effects. Principles of molecular docking analyses are inherently ethical, as they avoid the use of animals for initial exploratory research (35). The design of experiments in this study aligns with ethical principles of minimizing harm to animals and adhering to the 3Rs (Replacement, Reduction, Refinement) in conducted *in vivo* research (35).

Our study compared the inhibitory effect of flavonoids (myricetin, luteolin, isorhamnetin, quercetin, apigenin, kaempferol) to phenolic acids (chlorogenic acid, neochlorogenic acid, and caffeic acid) toward ADA enzyme. It provides information on binding energies and dissociation constants of flavonoids-enzyme complexes for luteolin, isorhamnetin, apigenin, and kaempferol. Uba et al. showed that phenolic compounds (chlorogenic acid, quercetin, and hyperoside) stabilized the ADA complex by forming persistent interactions with the catalytically essential Zn^{2+} ion (36). Our study did not reveal any interaction between the compounds and the zinc atom, however, it revealed hydrogen bonding between the 7-hydroxy group and Leu 62 residue (Figure 1) in all flavonoids. Compared to other flavonoids investigated, myricetin has an additional hydroxy group that forms an additional H-bond at a 5' position with the Gly 148 residue (Figure 1A). In addition to the binding energies, the values of the dissociation constant dictate the efficacy of binding of the polyphenols with the protein. The lower the dissociation constant, the lower the concentration of the compound is needed to stabilize the complex. Following the presented results, flavonoids are selected as more potent ADA inhibitors in comparison to phenolic acids.

Furthermore, chlorogenic acid exhibited a binding energy score ($\Delta G = -18.76 \pm 4.60$ kcal/mol) comparable to those of the two approved ADA inhibitor drugs pentostatin ($\Delta G = -14.54 \pm 2.25$ kcal/mol) and cladribine ($\Delta G = -25.52 \pm 4.10$ kcal/mol), while quercetin was found to have modest binding affinity ($\Delta G = -8.85 \pm 7.32$ kcal/mol). The results of our study are additional confirmation of the possible inhibitory potential of phenolic compounds against ADA compared to a prior study (36). Furthermore, our study is positively comparable with the first studies done on the ADA enzyme *in vitro*, which demonstrated that natural substances such as quercetin, myricetin, and kaempferol were able to inhibit the deamination of adenosine (37,38). Li et al. investigated the inhibitory effects of natural substances kaempferol, quercetin, myricetin, naringenin, and naringin, on the ADA-catalyzed deamination of cordycepin. This study confirmed good binding affinity for ADA enzyme, directly selecting them as particularly good competitive substrates of cordycepin (39).

Nowadays, the treatment of RA typically involves the chronic use of chemical medicines, such as NSAIDs,



AQ4 **FIGURE 1.** Binding modes of the tested flavonoids at the active site of murine ADA as assessed by molecular docking study (A) Myricetin. (B) Luteolin. (C) Isorhamnetin. (D) Quercetin. (E) Apigenin. and (F) Kaempferol.

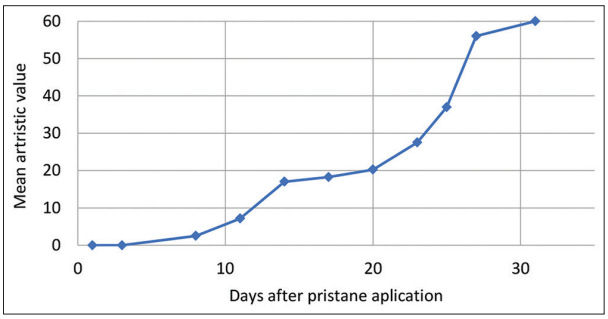


FIGURE 2. Mean arthritis score of PIA rat model after pristane application.

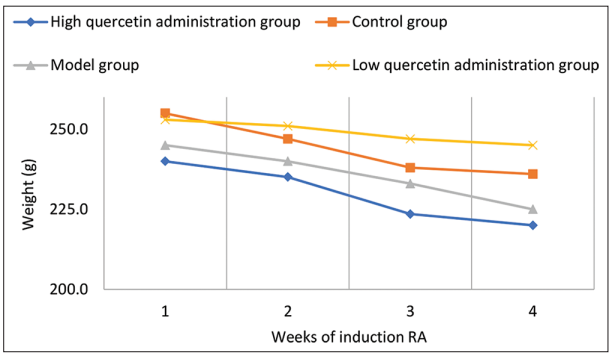


FIGURE 3. Body weight of PIA rat model after pristane application.

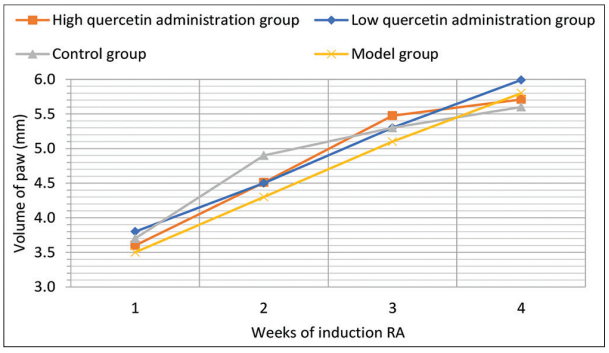


FIGURE 4. The volume of the hind paw of the PIA rat model after pristane application.

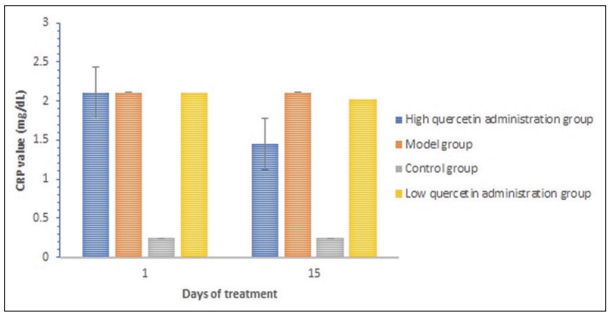


FIGURE 5. Serum CRP levels in high quercetin administration group, low quercetin administration group, model group, and control group.

DMARDs, and corticosteroids. These medications are designed to delay structural deformities in RA or control pain. However, they are associated with severe side effects and high costs. Polyphenols, natural phytocompounds, have demonstrated superior anti-inflammatory effects and represent a plentiful natural herbal resource (40).

The pristane-induced rat model has been widely recognized as a valuable tool in studying inflammatory processes, particularly in the context of conditions like RA. Pristane is an immune-boosting agent, and its injection can induce a condition reminiscent of autoimmune disorders, making it a suitable model for evaluating the anti-inflammatory properties of various compounds, including polyphenols (31). In the presence of inflammation, serum CRP is associated with the onset and progression of arthritis. It plays a part in the progression of RA (41).

The choice of the pristane-induced rat model is rooted in its ability to closely mimic the characteristics of human RA, including synovial inflammation, joint damage, and autoimmune responses. The model offers a controlled and reproducible environment in which to assess the anti-inflammatory potential of polyphenols (42).

In the pristane-induced rat model, researchers can explore the effects of different polyphenols on disease progression. Parameters such as paw swelling, inflammatory cytokine levels, and histological changes in joint tissues can be examined to assess the anti-inflammatory potential of these compounds (31). Studies utilizing this model have revealed that certain polyphenols can effectively reduce inflammation, alleviate symptoms, and slow disease progression.

The results of our study suggest that Wistar rats exhibit disease

onset 2 weeks after induction, correlating with the results of previous studies (31,40). Wang et al. state that PIA is a suitable model for the development of severe inflammation and edema in rats, whose symptoms persist for 2-3 weeks, which was also the case during the induction of our model (42).

Several studies have reported that the development of PIA is associated with the secretion of cytokines, which were significantly increased during disease progression (43,44).

Previous studies have demonstrated that QUE is a potential agent for the treatment of RA; however, the underlying anti-arthritic mechanism of QUE has not been fully elucidated (45-47).

The present study addressed the molecular docking and biochemical activities of quercetin through the ADA enzyme in an arthritic rat model. Quercetin has shown favorable binding to the ADA enzyme and selected flavonoids for *in vivo* testing.

When arthritic rats were compared with their normal control counterparts, they showed signs of arthritis. In contrast to their arthritic control counterparts, the model group of arthritic rats reported a marked decrease in body weight and a marked increase volume of paws. These results are consistent with that of other studies (48,49).

Our results showed that after 15 days, QUE had a significantly decreased CRP in the observation group of the pristane-induced rat model, this was consistent with previous studies of the anti-inflammatory effect of quercetin administrated alone and in combination with MTX (50).

Our results are consistent with other models of RA, where oral administrated 100 mg/kg b.w. of quercetin for 21 days lowered the level of CRP (51).

It is known that the anti-inflammatory effect of flavonoids and phenolic acid can be achieved through different mechanisms: inhibition of pro-inflammatory enzymes, modulation of immune response, and scavenging free radicals (52-54).

These findings suggest that polyphenols could be considered potential therapeutic agents for managing inflammatory conditions, including RA. While the pristane-induced rat model provides valuable insights into the anti-inflammatory potential of polyphenols, it's essential to recognize its limitations. The model is a simplification of complex human diseases, and the translation of results to clinical applications requires further research (55).

Incorporating *in silico* studies into research before moving on to *in vivo* experiments is a strategic and ethical approach that can enhance the quality and efficiency of research. It allows for more informed decisions and optimization of experimental parameters, potentially leading to more successful outcomes. *In silico* studies involve computer-based simulations and modeling to investigate the interactions between molecules, such as polyphenols, and enzymes like ADA. These studies are valuable for predicting how various compounds might affect enzyme activity without the need for extensive laboratory experiments (56).

LIMITATIONS OF THE STUDY

The PIA model in rats is a useful tool for studying aspects of RA; however, it also has several limitations. One key limitation is that the pathology of PIA does not fully recapitulate the complexity of human RA, as it primarily involves synovitis without the systemic manifestations seen in RA patients. In addition, the timing and severity of arthritis development can vary, making it challenging to control experimental outcomes. Furthermore, the immune response induced by pristane differs from the autoimmune mechanisms involved in human RA, which may limit the translational relevance of findings from this model to human disease (57).

CONCLUSION

The interplay between flavonoids, phenolic acids, and the ADA enzyme is a fascinating field of study with significant implications for health and disease. The *in silico* approach provides a valuable starting point for this investigation and holds promise for the development of novel therapeutic strategies in the future. The pristane-induced rat model serves as a valuable platform for evaluating the anti-inflammatory potential of polyphenols, offering insights into their effects on inflammatory processes to disease. Quercetin, a selected polyphenol according to *in silico* studies, at a dose of 400 mg/kg, showed statistically significant efficacy in reducing inflammation caused by pristane injection. While promising, further research is necessary to harness the full therapeutic potential of these natural compounds in the management of inflammatory disorders.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science, Higher Education and Youth of Sarajevo Canton, Sarajevo, Bosnia and Herzegovina (Project number: 27-02-11-41250-1/21). The authors acknowledge resources and support from the Zevet Veterinary Station, Zenica, Bosnia and Herzegovina.

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