Neoplasma 2023; 70(4): 545–554

doi:10.4149/neo_2023_230405N186

The effects of *Rhus coriaria* L. (sumac) and *Cinnamomum zeylanicum* L. bark on serum cytokine levels in rat mammary carcinogenesis

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Received April 5, 2023 / Accepted August 14, 2023

The purpose of this study was to assess the potential effects of *Rhus coriaria* L. (sumac) and of *Cinnamomum zeylanicum* L. bark on the selected serum cytokines as possible serum tumor markers – interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α) in the rat model of mammary carcinogenesis. *R. coriaria* and *C. zeylanicum* bark were used as the chemopreventive-therapeutic agents taken by rats in the powder form in the diet at two different concentrations during the entire period of two experiments carried out separately: lower concentration 1 g/kg – 0.1% and higher concentration 10 g/kg – 1%. The serum levels of cytokines of IL-6, IL-10, and TNF-α were determined using an enzyme-linked immunosorbent assay. In the first experiment treated with *R. coriaria*, a significant decrease in serum levels of IL-6 and TNF-α was present at higher concentrations compared to the chemoprevention-free control group. *R. coriaria* at lower concentrations non-significantly reduced the serum levels of IL-6 and TNF-α when compared to controls. A significant decrease in serum levels of TNF-α was present at higher concentrations compared to lower concentrations. The significant effect of *R. coriaria* on the serum levels of IL-10 was not observed. In the second experiment treated with *C. zeylanicum* bark, a significant decrease in serum levels of IL-6 was observed in lower and higher concentrations compared to the chemoprevention-free control group. *C. zeylanicum* bark non-significantly reduced the serum levels of TNF-α and had no effect on the serum levels of IL-10. In conclusion, *R. coriaria* and *C. zeylanicum* bark demonstrated significant anti-inflammatory effects by analyzing the selected serum cytokine levels in the rat breast carcinoma model. Observed anti-inflammatory effects of both plant-natural substances were associated with their anticancer activities in rats.

Key words: Cinnamomum zeylanicum; cytokines; mammary carcinogenesis; rat; Rhus coriaria; sumac

Cytokines represent a large group of low-molecular, highly inducible, secretory proteins produced by various cells, primarily immunocompetent, in response to specific antigenic stimuli and non-specific mitogenic stimuli. They influence many different cells (e.g., cells of the immune system, nervous system, endocrine system) directly through specific cell surface receptors with high affinity specific for a given cytokine. In their autocrine and/or paracrine action, mutual interaction is applied, resulting in synergic, antagonistic, or additive effects on cells.

Cytokines have great biological potency even at low concentrations, ranging from nanograms to picograms. In physiological concentrations, they play a positive role in immune response, coordinate its course, and protect against inflammation, infection, and tissue damage [1, 2]. On the other hand, their long-term and excessive production can lead to dysregulation of the immune response, harm to the organism, and promotion and development of diseases, including oncological ones [1, 2]. Thus, some cytokines have been closely related to tumorigenesis, as well as to the initiation, promotion, angiogenesis, and metastasis of breast cancer. In these cases, inflammation has been considered a prominent factor [1, 3, 4].

However, the exact initiation process of cancer including breast cancer as well as cancer metastasis is unknown. Tumor development in the forms of cancer initiation, growth, migration, metastasis, and recurrence is determined not only by genetic and epigenetic changes but also by interactions between tumor cells and non-transformed cells such as fibroblasts, mesenchymal stem cells, endothelial cells, pericytes, and also immune cells (macrophages and lymphocytes) present in the tumor microenvironment (TME) [5, 6]. The TME promotes the secretion of various cytokines and generates chronic inflammation. Persistent production of cytokines stimulates immune cells to secrete more cytokines and thus support tumor development, as in the case of breast cancer [7].

Current preclinical research has shown the beneficial action of phytochemicals and whole plant foods on multiple cancer-related biological pathways [8-13] to reduce the risk of breast cancer [14-17]. In our previous studies, *R. coriaria* demonstrated significant oncostatic activities and *C. zeylanicum* bark showed chemopreventive and therapeutic activities in the experimental animal model of breast cancer that were confirmed by mechanistic *in vivo* and *in vitro* studies [18, 19].

Sumac (also known as sumach) is a burgundy-purple coarsely ground powder from the dried berries of the *R. coriaria* plant (also commonly called Sicilian sumac, tanner's sumac, or elm-leaved sumac). *R. coriaria* grows in subtropical and temperate climates, in the areas of the Mediterranean, Asia, and Africa. Sumac is used as a spice in Mediterranean and Middle Eastern cuisines. It is also available as an herbal supplement in the form of capsules, herbal mixture, and tincture, and has been used in traditional medicine for centuries. It is a rich source of fiber, unsaturated acids such as oleic acid and linoleic acid, and some vitamins such as C, B6, B1, and B2. It also contains a high proportion of antioxidants such as tannins, anthocyanins, and flavonoids [20], which have a protective, anti-inflammatory, and anti-tumor effect on cells. It demonstrates high antioxidant activity [21] and therapeutic potential in cancer [22].

Cinnamon is a clean, dried young inner bark obtained from several species of trees of the genus *Cinnamon*. There are several types of cinnamon, while Ceylon cinnamon ("true cinnamon" from Sri Lanka) from the species *Cinnamomum zeylanicum* L. (also known as *Cinnamomum verum* L.) has the most antioxidant and health-promoting effects, being a rich source of fiber and minerals, mainly calcium, manganese, and iron. Furthermore, cinnamon is an important spice in many cuisines as part of some foods and drinks due to its distinctive taste and smell. It is also available in the form of nutritional supplements. With its significant antioxidant effect [23], mainly caused by the polyphenols present in cinnamon, it protects cells from oxidative stress, susceptibility to inflammation, and many diseases, including cancer.

Based on these findings, we have focused on the effects of *R. coriaria* and *C. zeylanicum* bark on serum cytokine levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) as possible serum tumor markers in tumor development of experimental rat mammary carcinogenesis.

Our research was based on assumptions and results from other studies on the role of cytokines in the initiation and progression of breast cancer, which is associated with numerous mechanisms of carcinogenesis such as proliferation, angiogenesis, invasiveness, and the formation of metastases, where inflammation and inflammation-induced cytokines play an important role [24]. This study is an extension of our previous experiments where sumac and cinnamon showed significant antitumor activities in a rat breast carcinoma model [18, 19], and we hypothesize that this is due to the anti-inflammatory activity of these natural foods.

Material and methods

Study design. In two separately running experiments, 45 intact female Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany) at 5 weeks of age were used. Animals were adapted to the standard conditions of the vivarium (artificial light regimen, light:dark/12:12 h, temperature 23±2 °C, and relative humidity 40-60%). The animals were on a low-phytoestrogen Ssniff® diet for rats (R-Z/M-Z; Soest, Germany) and water *ad libitum*. The N-methyl-N-nitrosourea (NMU) as a carcinogen (Sigma, Deisenhofen, Germany) was used to induce mammary carcinogenesis. NMU was dissolved in saline (0.5 ml/animal) and then injected intraperitoneally on the 42nd postnatal day at a dose of 50 mg/kg of the animal's body weight. These models mimicked conditions in healthy but high-risk premenopausal women for the development of breast cancer.

Experiment with *R. coriaria*. *R. coriaria* was used as a chemopreventive-therapeutic agent taken by rats in the powder form in the diet (SONNENTOR Kräuterhandels GMBH, Sprögnitz, Austria; country of origin-Iran). Chemoprevention began 7 days before carcinogen administration and lasted until the end of the experiment, i.e., 14 weeks. The animals were divided into 3 groups (15 animals per group). Group 1-the control (CONT) group was without chemoprevention; groups 2 and 3 were treated with *R. coriaria* chemoprevention at different concentrations-group 2 at a lower concentration 1 g/kg-0.1% (SUM 0.1) and group 3 at a higher concentration 10 g/kg-1% (SUM 1).

Experiment with *C. zeylanicum*. *C. zeylanicum* bark was used as a chemopreventive-therapeutic agent taken by rats in the powder form in the diet (Calendula, Nova Lubovna, Slovak Republic; country of origin-Indonesia). Chemoprevention began 7 days before carcinogen administration and lasted until the end of the experiment, i.e., 14 weeks. The animals were divided into 3 groups (15 animals per group). Group 1-the control (CONT) group was without chemoprevention; groups 2 and 3 were treated with *C. zeylanicum* bark chemoprevention at different concentrations-group 2 at a lower concentration 1 g/kg-0.1% (CIN 0.1) and group 3 at a higher concentration 10 g/kg-1% (CIN 1).

Animal experiments, cytokine analyses. Once a week, the rats were weighed and palpated. Mammary tumors were evaluated in terms of their presence, numbers, location, and size. At the end of the experiments, the animals were killed by rapid decapitation. Blood samples were collected into a serum separator tube. After clot formation, the samples were centrifuged at 2,000×g for 10 min and serum was collected. Serum samples were assayed to assess serum levels of the following cytokines: IL-6, IL-10, and TNF-α (Department of Microbiology and Immunology, Jessenius Faculty of Medicine in Martin, Comenius University Bratislava). Serum levels of cytokines were determined using the following ELISA *in vitro* kits designed for quantitative measurement of cytokines in rat serum: Rat IL-6 *in vitro* ELISA kit, Rat IL-10 *in vitro* ELISA kit, and Rat TNF-α *in vitro* ELISA kit (Abcam ELISA kits, Biotech s.r.o. Bratislava).

Ethical statement. All procedures were carried out according to EU directives, reviewed by the Ethics Committee of the Jessenius Faculty of Medicine of Comenius University (Protocol No. EK1860/2016) and by the State Veterinary and Food Administration of the Slovak Republic (accreditation No. Ro-3239/15-221 and Ro-1640/17-221).

Data analyses. Data were explored and analyzed in R [https://www.r-project.org/], ver. 4.0.5. Boxplot, overlaid with swarmplot and quantile-quantile plot with 95% confidence band constructed by bootstrap were used to assess normality of the data. A symbox plot was used to explore the transformations needed to bring data closer to normality. Concentrations of cytokines were log-transformed in the experiment with *R. coriaria* and not transformed in the experiment with *C. zeylanicum* bark. Multivariate ANOVA (MANOVA) was used to model the differences between groups (CONT, SUM 0.1/CIN 0.1, SUM 1/CIN 1) in terms of the cytokines. Univariate and multivariate normality, outliers, multicollinearity, linearity assumption, and homogeneity of variance-covariance were assessed in the usual way. Pillai test was used to test the Manova hypothesis, followed by the post-hoc univariate ANOVAs, where appropriate. Pairwise comparisons were performed using Games-Howell post-hoc tests. Differences between groups were considered significant when $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$.

Results

Effect of *R. coriaria* treatment on serum cytokines levels. In the first experiment treated with *R. coriaria*, a significant effect of *R. coriaria* on the serum levels of cytokines IL-6 and TNF-α in the experimental model of breast carcinoma in female Sprague-Dawley rats was observed. Concerning IL-6, *R. coriaria* significantly decreased its serum levels in the group SUM 1 ($^{**}p<0.01$) and non-significantly in the group SUM 0.1 compared to the CONT group, showing the dose-dependent link. However, between the SUM 1 and the SUM 0.1 groups, no significant difference in the serum levels of IL-6 was observed (Figure 1). In the case of TNF-α, *R. coriaria* led to a significant decrease of its serum levels in the group SUM 1 ($^{****}p<0.0001$) and a non-significant decrease in the group SUM 0.1 compared to the CONT group, showing the dose-dependent link. Between the SUM 1 and the SUM 0.1 groups a significant dose-dependent difference ($^{***}p<0.001$) in the serum levels of TNF-α was shown (Figure 2). The effect of *R. coriaria* in both SUM 0.1 and SUM 1 groups on the serum levels of IL-10 was not significant (Figure 3). The summary statistics of log-transformed cytokines in the experiment with *R. coriaria* is included in Table 1.

Effect of *C. zeylanicum* in serum cytokine levels. In the second experiment treated with *C. zeylanicum* bark, a significant effect of *C. zeylanicum* bark on the serum levels of cytokine IL-6 in the experimental model of breast carcinoma in female Sprague-Dawley rats was observed. Regarding IL-6, *C. zeylanicum* bark significantly decreased its serum levels in the CIN 0.1 group ($^{*}p<0.05$) and even more significantly in the CIN 1 group ($^{***}p<0.001$) when compared to the CONT group, showing the dose-dependent link. Non-significant difference of *C. zeylanicum* bark on the serum levels of IL-6 was detected between the CIN 0.1 and the CIN 1 groups (Figure 4). In the case of the serum levels of TNF-α, these were non-significantly reduced by *C. zeylanicum* bark in

Table 1. Summary statistics of log-transformed cytokines in the experiment with *Rhus coriaria* L. (sumac).

Group	Variable	n	mean	median	sd	iqr
CONT	logcytokine IL-6	13	4.36	4.36	0.06	0.07
CONT	logcytokine TNF-α	14	4.77	4.78	0.08	0.10
CONT	logcytokine IL-10	14	4.92	4.85	0.27	0.29
SUM 0.1	logcytokine IL-6	15	4.32	4.30	0.07	0.06
SUM 0.1	logcytokine TNF-α	15	4.70	4.68	0.19	0.15
SUM 0.1	logcytokine IL-10	15	4.93	4.89	0.27	0.27
SUM 1	logcytokine IL-6	15	4.28	4.26	0.04	0.07
SUM 1	logcytokine TNF-α	15	4.45	4.45	0.09	0.04
SUM 1	logcytokine IL-10	14	5.05	4.80	0.46	0.58

Notes: CONT-control group without sumac administered; SUM 0.1-group with sumac administered at a lower concentration of 1 g/kg–0.1% in the diet; SUM 1-group with sumac administered at a higher concentration of 10 g/kg–1% in the diet

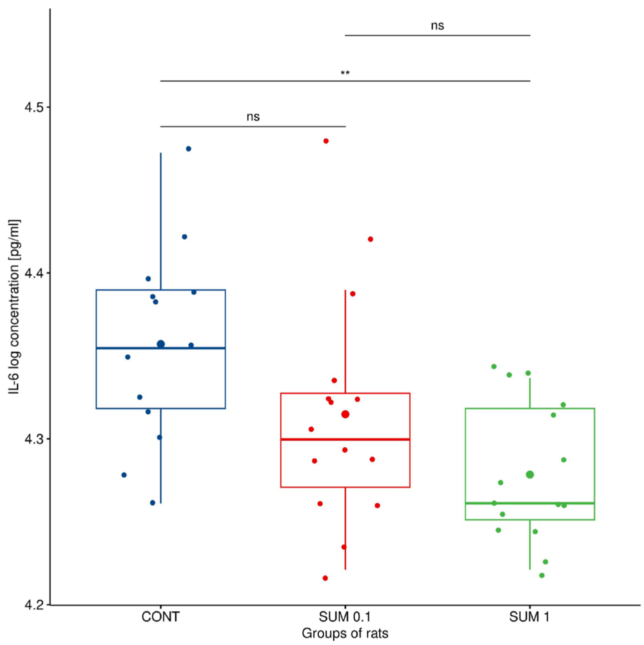

Figure 1. Serum levels of IL-6 cytokine after *Rhus coriaria* L. (sumac) treatment in experimental female rats' model of breast carcinoma induced by N-methyl-N-nitrosourea. Notes: CONT-control group without sumac administered; SUM 0.1-group with sumac administered with a lower concentration of 1 g/kg–0.1% in the diet; SUM 1-group with sumac administered with a higher concentration of 10 g/kg–1% in the diet. **p<0.01 vs. the CONT group

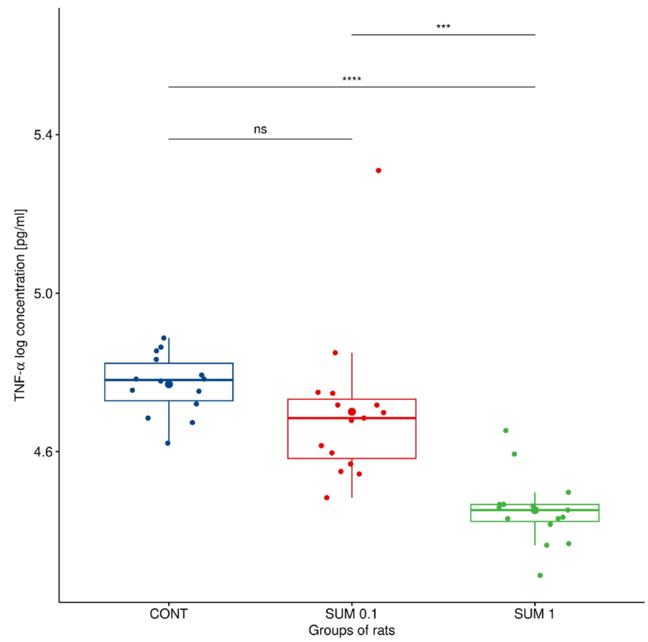

Figure 2. Serum levels of TNF-α cytokine after *Rhus coriaria* L. (sumac) treatment in experimental female rats' model of breast carcinoma induced by N-methyl-N-nitrosourea. Notes: CONT-control group without sumac administered; SUM 0.1-group with sumac administered at a lower concentration of 1 g/kg–0.1% in the diet; SUM 1-group with sumac administered at a higher concentration of 10 g/kg–1% in the diet. ****p<0.0001 vs. CONT group and ***p<0.001 vs. SUM 0.1 group

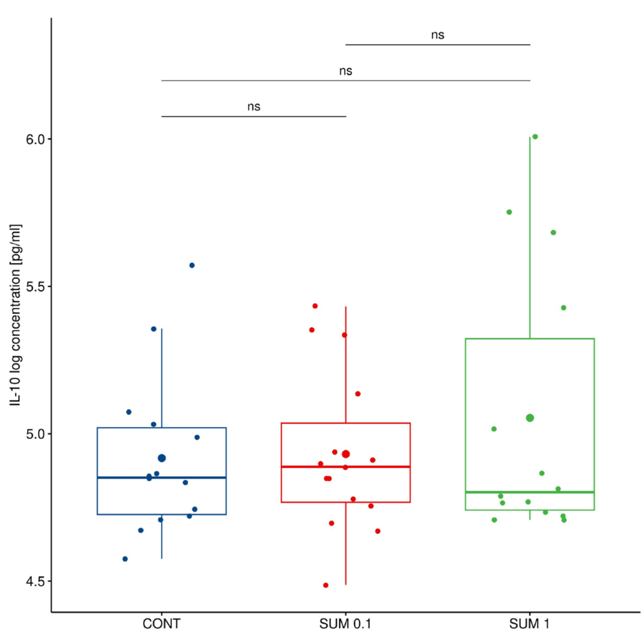

Figure 3. Serum levels of IL-10 cytokine after *Rhus coriaria* L. (sumac) treatment in experimental female rats' model of breast carcinoma induced by N-methyl-N-nitrosourea. Notes: CONT-control group without sumac administered; SUM 0.1-group with sumac administered at a lower concentration of 1 g/kg–0.1% in the diet; SUM 1-group with sumac administered at a higher concentration of 10 g/kg–1% in the diet

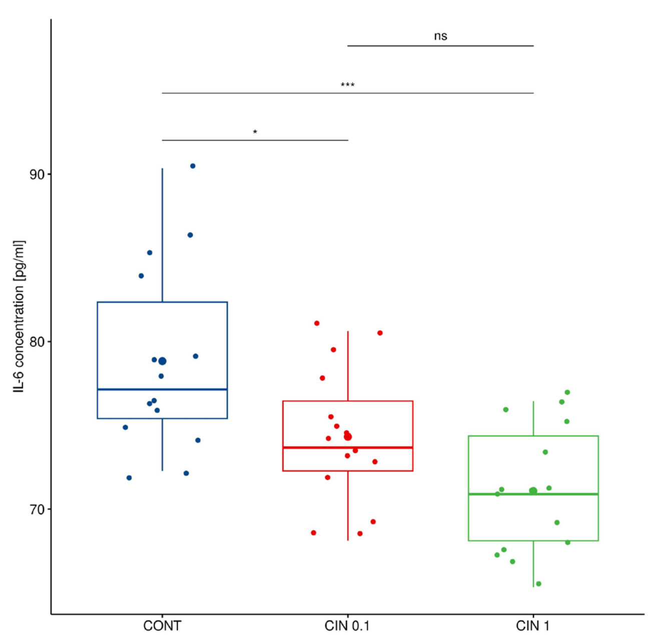

Figure 4. Serum levels of IL-6 cytokine after *Cinnamomum zeylanicum* L. bark treatment in experimental female rats' model of breast carcinoma induced by N-methyl-N-nitrosourea. Notes: CONT-control group without *C. zeylanicum* L. bark administered; CIN 0.1-group with *C. zeylanicum* L. bark administered at a lower concentration of 1 g/kg–0.1% in the diet; CIN 1-group with *C. zeylanicum* L. bark administered at a higher concentration of 10 g/kg–1% in the diet. ***p<0.001 vs. CONT group and *p<0.05 vs. CONT group

both the CIN 0.1 and the CIN 1 groups when compared to the CONT group (Figure 5). *C. zeylanicum* bark had no effect on the serum levels of IL-10 in both the CIN 0.1 and the CIN 1 groups (Figure 6). In Table 2, the statistics of cytokines in the experiment with *C. zeylanicum* bark is summarized.

Discussion

In the previous experiments, the chemopreventive and therapeutic effects of various plant sources were found in the experimental breast carcinoma model [18, 19, 25-30]. *R. coriaria* oncostatic activities and chemopreventive-therapeutic efficacy of *C. zeylanicum* bark in the experimental model of breast carcinoma induced by NMU have been demonstrated [18, 19]. The present experimental study aims to further demonstrate the potential effects of *R. coriaria* and the *C. zeylanicum* bark on serum cytokine levels of IL-6, IL-10, and TNF-α in rat mammary carcinogenesis.

Overexpression of inflammatory cytokines, including IL-6 and TNF-α, plays crucial roles in tumorigenesis of various cancers. The targeting signaling inflammatory pathways, such as Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and nuclear factor-κB (NF-κB), are important in promoting inflammatory breast cancer [3,

Table 2. Summary statistics of cytokines in the experiment with *Cinnamomum zeylanicum* L. bark.

Group	Variable	n	mean	median	sd	iqr
CONT	cytokine IL-6	14	78.8	77.1	5.5	7.0
CONT	cytokine TNF-α	14	84.3	83.4	14.4	18.3
CONT	cytokine IL-10	14	158.6	155.1	46.7	47.9
CIN 0.1	cytokine IL-6	15	74.3	73.7	4.0	4.2
CIN 0.1	cytokine TNF-α	15	81.2	76.7	17.1	11.7
CIN 0.1	cytokine IL-10	15	141.6	142.3	34.7	51.3
CIN 1	cytokine IL-6	15	71.1	70.9	3.8	6.3
CIN 1	cytokine TNF-α	15	78.5	80.1	8.5	13.3
CIN 1	cytokine IL-10	15	158.9	150.5	50.8	59.5

Notes: CONT-control group without *C. zeylanicum* L. bark administered; CIN 0.1-group with *C. zeylanicum* L. bark administered at a lower concentration of 1 g/kg–0.1% in the diet; CIN 1-group with *C. zeylanicum* L. bark administered at a higher concentration of 10 g/kg–1% in the diet

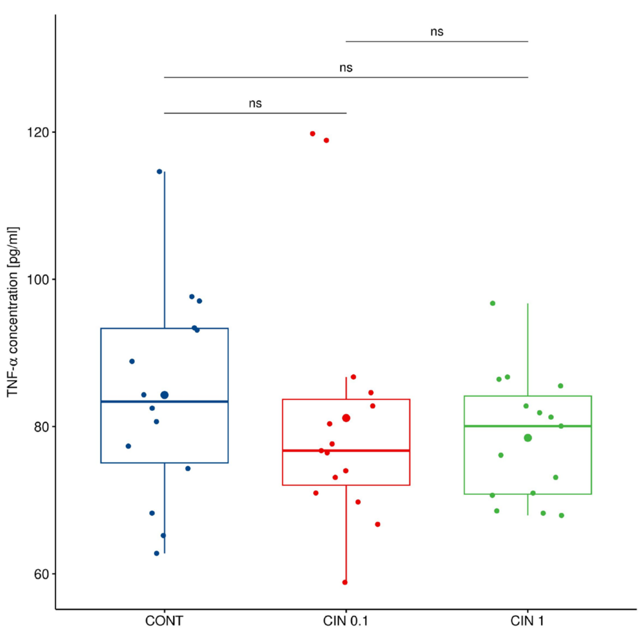

Figure 5. Serum levels of TNF-α cytokine after *Cinnamomum zeylanicum* L. bark treatment in experimental female rats' model of breast carcinoma induced by N-methyl-N-nitrosourea. Notes: CONT-control group without *C. zeylanicum* L. bark administered; CIN 0.1-group with *C. zeylanicum* L. bark administered at a lower concentration of 1 g/kg–0.1% in the diet; CIN 1-group with *C. zeylanicum* L. bark administered at a higher concentration of 10 g/kg–1% in the diet

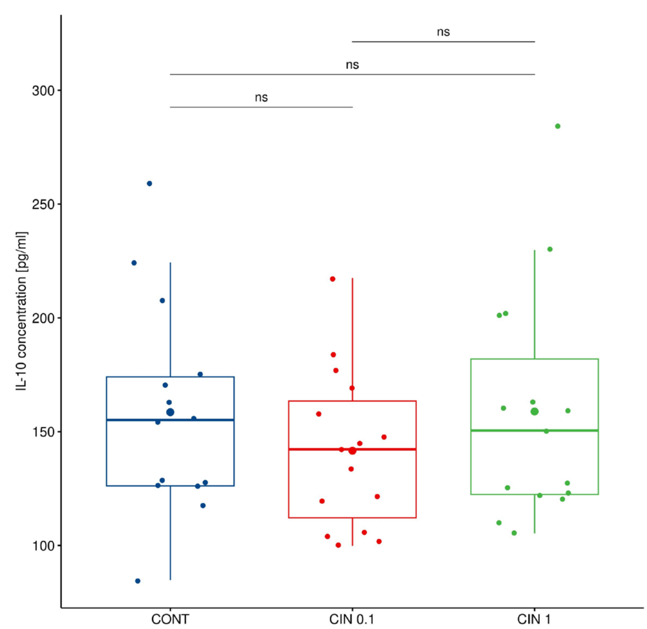

Figure 6. Serum levels of IL-10 cytokine after *Cinnamomum zeylanicum* L. bark treatment in experimental female rats' model of breast carcinoma induced by N-methyl-N-nitrosourea. Notes: CONT-control group without *C. zeylanicum* L. bark administered; CIN 0.1-group with *C. zeylanicum* L. bark administered at a lower concentration of 1 g/kg–0.1% in the diet; CIN 1-group with *C. zeylanicum* L. bark administered at a higher concentration of 10 g/kg–1% in the diet

31]. These pathways contribute to many cellular processes, including the regulation of immune and inflammatory responses, cellular growth, and apoptosis [32]. The mechanism of *R. coriaria* L. effects appears to be through inhibiting inflammatory pathways NF-κB, STAT3, and nitric oxide [22].

IL-6 is a pleiotropic pro-inflammatory cytokine with a wide variety of physiological functions, such as hematopoiesis, steroidogenesis, and neurotransmission, and is produced primarily by monocytes/macrophages, T lymphocytes, and endothelial cells [33, 34]. IL-6 has been reported to activate the JAK/STAT pathway. As a major mediator of inflammation, it induces the expression of STAT3 target genes and drives tumor growth and/or survival. IL-6/JAK2/STAT3 signaling can reduce anti-tumor immunity of tumor-infiltrating immune cells [3]. IL-6 is one of the pro-inflammatory cytokines regulated by the NF-κB pathway and is hyperactivated in breast cancer. Suppression of NF-κB pathway signaling reduces the secretion of pro-inflammatory cytokines, including IL-6, which could inhibit inflammatory breast cancer tumor growth, motility, and angiogenesis [3]. Several studies have shown a relationship between serum IL-6 levels and breast cancer progression, suggesting the role of a negative prognostic marker [35, 36]. Serum levels of IL-6 are significantly higher in women with breast cancer than in healthy women. Increased levels of IL-6 have been shown to decrease response to endocrine hormone therapy and chemotherapy and correlate with a poor prognosis and worse survival [36-40]. On the contrary, lower serum levels of IL-6 correlate with a better response to therapy and a good prognosis [40, 41]. IL-6 is a critical factor in the development of breast cancer metastasis. In this sense, IL-6 cytokine could be considered a prognostic marker and a therapeutic target for this disease [42]. The study by Wang and Yang [43] has also reported that serum IL-6 levels may serve as a useful indicating tool when assessing the stage of breast cancer - the higher serum IL-6 levels, the more advanced stage, including the presence of metastasis. On the other side, the declined serum IL-6 levels were recorded in patients with proven responses to the treatment.

In the first experiment, *R. coriaria* at a higher dose significantly decreased serum IL-6 levels in rats, and at a lower dose non-significantly reduced serum IL-6 levels compared to the CONT group. This result may be related to the significant reduction in tumor incidence in rats in the group of *R. coriaria* at a higher dose and a non-significant decrease in tumor incidence at a lower dose [18]. A dose-dependent decrease in high-/low-grade carcinoma ratios was observed from histopathological analysis of *R. coriaria*-treated groups [18]. The other parameters of rat mammary carcinogenesis, such as tumor frequency, tumor latency, and average tumor volume, did not change significantly in *R. coriaria*-treated rat groups compared to the CONT group [18]. It has been proven that *R. coriaria* extract significantly reduced IL-6 production levels in the MDA-MB-231 breast cancer cell line in a dose-dependent manner. Data suggest that downregulation of the inflammatory cytokine IL-6 could inhibit tumor growth and metastasis mediated by *R. coriaria* [22].

In the second experiment, *C. zeylanicum* bark significantly decreased IL-6 serum levels in rats treated with both, higher and lower doses compared to the CONT group. This significant decrease in serum levels of IL-6 could be related to a significantly reduced tumor incidence in rats in both *C. zeylanicum* bark-treated groups [19]. A significant decrease in the ratio of high-/low-grade carcinomas of tumor histopathology was shown in both *C. zeylanicum* bark-treated groups [19]. The other parameters of rat mammary carcinogenesis, such as decreased tumor frequency and lengthening of tumor latency, were of boundary significance in groups treated with *C. zeylanicum* bark [19]. The bark of *C. zeylanicum* significantly reduced tumor volume at a higher dose compared to the group with a lower dose [19]. This result could correspond to the anti-inflammatory effects of cinnamon in this study associated with the significant decrease in serum levels of IL-6 in the treated group at a higher dose of *C. zeylanicum* bark.

TNF-α is a pro-inflammatory cytokine, produced by a number of cell populations such as monocytes/macrophages, T lymphocytes, mastocytes, granulocytes, NK cells, fibroblasts, neurons, keratinocytes, as well as smooth muscle cells [44]. It occurs in the body in two forms, namely the transmembrane form (*tm*TNF-α) and the free/soluble form (*s*TNF-α) [45], which initiate a signaling cascade leading to apoptosis or NF-κB activation. Activation of NF-κB leads to the expression of pro-inflammatory genes with resulting stimulation of the inflammatory response, as well as increased expression of cytokines, including TNF-α and cell proliferation [44]. TNF-α is involved in a number of processes in the body, which can also contribute to the development of serious pathological conditions, including tumors in the case of impaired regulation of its secretion [46]. TNF-α is highly expressed in breast cancers [47]. Similarly, as in the case of serum IL-6 levels, the higher serum TNF levels correlate with higher tumor stage, including the presence of metastasis [48-50].

In the first experiment, the effect of *R. coriaria* on the serum levels of TNF-α in rats was more significant than its effect on the serum levels of IL-6. *R. coriaria* at a higher dose led to a significant decrease and at a lower dose to a non-significant decrease of the serum levels of TNF-α when compared to the CONT group. This result seems to show a link between the significant reduction of tumor incidence in rats in the group of *R. coriaria* at a higher dose and a non-significant decrease in tumor incidence at a lower dose [18]. A significant difference in the decrease of TNF-α was observed also between the treated groups. The data have shown that *R. coriaria* extract induced a marked reduction of TNF-α in the MDA-MB-231 breast cancer cell line in a dose-dependent manner. The data suggest that downregulation of inflammatory cytokines such as TNF-α could inhibit tumor growth and metastasis mediated by *R. coriaria* [22].

In the second experiment, the serum levels of TNF-α were non-significantly reduced by *C. zeylanicum* bark in both treated groups when compared to the CONT group.

IL-10, originally referred to as "cytokine synthesis inhibitor factor", is predominantly an anti-inflammatory cytokine, produced by a variety of cell types, but primarily by monocytic cells [51]. Its primary function is to facilitate immune responses and dampen inflammation, also by downregulating pro-inflammatory cytokine secretion (e.g., TNF-α, IL-6, etc.) [52]. As a result, it may be a factor that influences anti-tumor and/or mucosal immune responses. However, in contrast, too much of the immunosuppressive activity of IL-10 can contribute to chronic infections associated with chronic inflammation. The immunomodulatory roles of IL-10 have not yet been fully explained, and both beneficial and detrimental roles of IL-10 have been observed in immune responses and disease pathology [42, 53]. In other words, IL-10 can exert an inhibitory effect on NF-kB activity and is also involved in JAK/STAT signaling pathway regulation [54]. IL-10 can induce STAT-3 activation [55], which also leads to induction of tumor cell proliferation [56, 58]. The production of IL-10 by tumor cells in the TME suppresses the anti-tumor activities of immune cells (NK, T, and B cells) in the TME, thus allowing the survival and proliferation of tumor cells [58-60]. In cancer cells, the expression of IL-10 was elevated, which can downregulate cancer cytokines and finally attenuate macrophage phagocytosis [61]. A study by Wang and Yang [43] showed that serum IL-10 concentration was significantly higher in patients with breast cancer at higher stages (TNM III and IV) compared to patients with breast cancer at lower stages (TNM I and II) and healthy control individuals. Furthermore, the expression of IL-10 showed a reduction in patients with responses after treatment. Similarly, another research by Kozlowski *et al.* [62] showed that serum levels of IL-10 are frequently higher in patients with breast cancer compared to healthy individuals. Elevated levels of IL-10 in serum could inhibit tumor growth by suppressing IL-6 production, confirming the inverse correlation between serum levels of IL-6 and IL-10 in cancer patients. However, higher levels of IL-10 correlate with metastatic breast cancer and a poor prognosis compared to nonmetastatic disease, which could result in impaired immunity, favoring tumor progression [1, 63]. The impact of increased levels of IL-10 on survival was not determined [62, 63]. Another study on breast cancer by Mohamed *et al.* [64] proved that IL-10 stimulates metastatic invasion into the lymphatic system. However, in contrast, another study by Li *et al.* [65] reported dissimilar results, showing that higher expression of IL-10 in breast cancer tumors was associated with a lower risk of metastasis. In both of our experiments, we did not confirm any significant effects of *R. coriaria* and *C. zeylanicum* bark on serum levels of IL-10.

Obesity, as one of the risk factors for breast cancer, is associated with elevated levels of proinflammatory cytokines and adipokines in tissues and circulation, which are produced by macrophages and adipocytes, and create a chronic inflammatory environment [66]. There is evidence that the risk of breast cancer decreases with intentional weight loss. The levels of TNF-α and IL-6 also decrease with intentional weight loss [67]. No significant differences in body weight gain were found in rats in both *R. coriaria* and *C. zeylanicum* bark experiments. However, a slight increase in the food intake of rats in *R. coriaria*-treated groups and a significant increase in the food intake of rats in the *C. zeylanicum* bark-treated groups compared to controls have been revealed [18, 19].

Diseases accompanied by chronic inflammation are very closely related to increased activation of the immune system, where cytokines also play a significant role and can participate in the pathological process by interfering with the regulatory mechanisms of the immune response. The role of cytokines in breast cancer development and progression is different. Some cytokines stimulate, while others inhibit breast cancer proliferation and/or invasion. Similarly, high circulation levels of some cytokines seem to be favorable, while others are unfavorable prognostic markers [68]. Today, many studies have been dealing with understanding the role of cytokines in the development of diseases including cancer diseases. Our research seems to have revealed a link between the reduced incidence of tumors and the reduced serum level of IL-6 and TNF-α in the group treated with *R. coriaria*, and of IL-6 in the group treated with *C. zeylanicum* bark. The scientific explanations of the role of serum cytokine levels, detected in the circulation, have not been fully elicitated and therefore it is highly important to further investigate its precise role as biomarkers to diagnose cancer, predict cancer outcomes, and manage therapeutic approaches in (breast) cancer patients.

Acknowledgments: This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic under Contract No. VEGA 1/0045/23.

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