Research Article

Sanguinarine-Chelerythrine Fraction of *Coptis chinensis* Exerts Anti-inflammatory Activity in Carrageenan Paw Oedema Test in Rats and Reveals Reduced Gastrotoxicity

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Received 25 November 2021; Revised 18 February 2022; Accepted 3 March 2022; Published 16 March 2022

Academic Editor: Javier Egea

Inflammatory diseases are a common therapeutic problem and nonsteroidal anti-inflammatory drugs are not deprived of side effects, of which ulcerogenic activity is one of the most frequent. The aim of the study was to evaluate the anti-inflammatory activity of the sanguinarine-chelerythrine (SC) fraction of *Coptis chinensis* and its influence on the integrity of gastric mucosa. The study was conducted on sixty male rats randomly divided into six experimental groups: two control groups (a negative control group CON and a positive control group CAR); three groups receiving an investigational fraction of *C. chinensis* (1, 5, 10 mg/kg i.g.) named SC 1, SC 5, and SC 10, respectively; and a group receiving indomethacin (IND) (10 mg/kg i.g.) as a reference drug. In all animals, the carrageenan-induced paw oedema was measured; PGE₂ release, TNF-α production, and MMP-9 concentration in inflamed tissue were determined. Additionally, the macroscopic and microscopic damage of gastric mucosa was evaluated. Administration of SC dose-dependently inhibited the second phase of carrageenan rat paw oedema and PGE₂ release, decreased the production of TNFα, and reduced the concentration of MMP-9, and the efficacy of the highest dose was comparable to the effect of IND. Contrary to IND, no gastrotoxic activity of SC was detected. The investigated sanguinarine-chelerythrine fraction of *C. chinensis* seems to be a promising candidate for further research on new anti-inflammatory and analgesic drugs characterized with a safer gastric profile compared to existing NSAIDs.

1. Introduction

Inflammation is an important defence mechanism protecting the organism against harmful stimuli, such as bacteria, viruses, and fungi. However, excessive inflammation is involved in the development of multiple diseases, e.g., destructive arthritis, inflammatory colitis, atherosclerosis, and diabetes mellitus. Chronic inflammatory diseases belong to the main problems affecting the lifestyle and quality of life of millions of people all over the world. However, existing therapies are not always effective and they are often associated with the burden of adverse effects that limit their usage. Nonsteroidal anti-inflammatory drugs (NSAIDs) are very efficient and widely used in the treatment of various inflammatory conditions, but gastrointestinal complications frequently restrict their application. In this context, there is a great need to look for new agents that, on one hand, would be effective in the management of pain and inflammation and, on the other hand, would be safer than existing ones. Natural compounds with anti-inflammatory activity are a
large and important group of pharmaceuticals that might be used in the treatment of various inflammatory diseases. Inflammatory stimuli (e.g. foreign organisms, tissue damage, or antigens) activate an immune response that under some conditions may be deleterious for the host and lead to chronic inflammation. The cell damage associated with inflammation acts on the cell membrane to release leukocyte lysosomal enzymes that liberate among others arachidonic acid (AA). Mobilized AA is then converted into a variety of prostanoids by means of cyclooxygenases 1 and 2 (COX-1 and COX-2). Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and prostacyclin (PGI\textsubscript{2}) are predominant prostanoids involved in inflammatory reactions. They enhance oedema by increasing vascular permeability and promoting leukocyte infiltration. They may regulate lymphocyte function, and PGE\textsubscript{2} with vascular permeability and promoting leukocyte infiltration in inflammatory diseases.

\textit{Coptis chinensis}, with its broad bioactive potential, is one of the 50 most important herbs in traditional Chinese medicine (TCM) [1]. TCM it has been used to treat various inflammatory diseases, high fever, toothache, gastrointestinal disorders, diabetes, and skin diseases for thousands of years [2]. Various contemporary studies confirmed the anti-inflammatory activity of \textit{C. chinensis} [3–6]. However, they attributed the anti-inflammatory properties of the investigated extract mainly to the activity of berberine, which is the most abundant alkaloid found in \textit{C. chinensis}.

Sanguinarine and chelerythrine (Figure 1) are benzophenanthridine alkaloids widely distributed in plants belonging to the \textit{Papaveraceae}, \textit{Fumariaceae}, \textit{Rutaceae}, \textit{Ranunculaceae}, and \textit{Meliaeae} families. Apart from \textit{C. chinensis}, these substances were also found as one of the most abundant compounds in \textit{Chelidonium majus} L. (CM) [2, 7–10]. Both alkaloids were also detected in other plant species, such as \textit{Sanguinaria canadensis}, a North American plant used as a dental antibacterial agent and as an antiarthritic and anticancer treatment [11].

Sanguinarine is a promising pharmacologically active substance of plant origin, because so far, in addition to its anti-inflammatory effect, its potential therapeutic use, e.g., in the treatment of osteoporosis (F. [12]), hypertension [13, 14], heart failure [15], atherosclerosis [16], cancer [17], infectious diseases (Q. [18, 19]), or asthma [20], has been reported. Very similar results were obtained with chelerythrine (H. [21]; N. [22–26]). Taking into account a considerable amount of reports confirming the positive effect of both alkaloids on various pathologies, the knowledge about the anti-inflammatory activity and possible use of these compounds in the treatment of inflammatory diseases should also be constantly expanded.

Zielińska et al. [27] reported that chelerythrine decreased the TNF\alpha secretion in human neutrophils and sanguinarine was the most potent inhibitor of IL-1\beta secretion among CM alkaloids. Other authors informed that anti-inflammatory activity of sanguinarine and chelerythrine may be a consequence of their inhibitory influence on the secretion of CCL-2, IL-6, and IL-1 RA [28]. Sanguinarine was also reported to be a potent inhibitor of the TLR4/NF\kappaB signal transduction [4].

The above promising reports prompted us to examine the potency and therapeutic application of these two alkaloids isolated from \textit{C. chinensis}. The aim of the study was to investigate the anti-inflammatory properties of sanguinarine-chelerythrine fraction isolated from \textit{C. chinensis} and its influence on the integrity of gastric mucosa.

2. Materials and Methods

2.1. Chemicals and Materials. The following drugs and chemicals were used to perform the experiment: carrageenan (Sigma-Aldrich, Steinheim, Germany) and indomethacin (Sigma-Aldrich, Steinheim, Germany); 0.9% saline solution (Polpharma, Starogard Gdański, Poland); ketamine and xylazine 20 mg/mL (Sedazin®, Biowet, Pulawy, Poland); ketamine 100 mg/mL (Biowet, Pulawy, Poland); and formalin 37% sol (Chempur, Piekary Slaskie, Poland). Other used chemicals were included in the commercially available kits.

2.2. Plant Compound Preparation. Sanguinarine-chelerythrine fraction of \textit{C. chinensis} extract was used in the carrageenan paw oedema test in rats. The fraction containing both alkaloids was isolated as a mixture (0.2 : 1 w/w) from \textit{C. chinensis} rhizoma (19 g/100 g yield) as described previously [10].

2.3. Animals. The study was conducted on sixty male Wistar rats (weighing 224.7 ± 10.0 g) purchased from the Animal Research Centre at Wrocław Medical University (Wrocław, Poland). Rats were housed in pairs in transparent polystyrene cages, under standard conditions of temperature (21-23°C), humidity (60–70%), and a light-dark cycle (12:12 h). Animals were fed a standard rodent diet (LSM, Agropol, Motycz, Poland), with access to food and water was ad libitum.

2.4. Ethics Statement. The study protocol was approved by the First Local Ethics Committee for Animal Experiments in Wrocław, Poland. All animal experiments were performed in accordance with ARRIVE guidelines and the EU Directive 2010/63/EU for animal experiments.

2.5. Drug Administration. Acclimated animals were randomized into six experimental groups of ten animals each: two control groups (a negative control group (CON) and a positive control group (CAR)) receiving 0.9% saline solution intragastrically (i.g.) (3 mL/kg), three groups receiving investigational fraction of \textit{C. chinensis} containing sanguinarine and chelerythrine (1, 5, and 10 mg/kg i.g. in saline solution 3 mL/kg) named SC\textsubscript{1}, SC\textsubscript{5}, and SC\textsubscript{10} respectively, and a group receiving indomethacin (IND) (10 mg/kg i.g. in saline solution 3 mL/kg) as a reference drug.

2.6. Carrageenan Paw Oedema Test. One hour after administration of the appropriate experimental substance, a single
Paw oedema in the individual animal was defined as a change of its paw volume:

\[
\text{Paw oedema} = \text{paw volume at the analyzed time point} - \text{initial paw volume.}
\]

The percentage of the inhibition of the inflammatory reaction was calculated with the following formula:

\[
\% \text{of inhibition} = \left(1 - \frac{\text{change of paw volume in the analyzed animal}}{\text{mean change of the paw volume in CAR group}}\right) \times 100.
\]

2.7. Isolation of Right Hind Paw. After euthanasia, the right hind paws were immediately cut off. The subplantar tissue was isolated and homogenized, and the obtained supernatant was frozen for further enzyme-linked immunosorbent assay (ELISA). The concentration of tumour necrosis factor alpha (TNFα), prostaglandin E₂ (PGE₂), and matrix metalloproteinase 9 (MMP-9) in the supernatant was assessed with a plethysmometer (Plethysmometer 37140 Ugo Basile, Gemonio, Italy) five times in each animal: before the injection of carrageenan/saline solution and 1, 2, 3, and 6 hours after the injection. After the last measurement, under deep anaesthesia with ketamine (60 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), rats were euthanized by dislocation of cervical vertebrae C6-C7.

Paw oedema in the individual animal was defined as a change of its paw volume:

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\text{Paw oedema} = \text{paw volume at the analyzed time point} - \text{initial paw volume.}
\]

The percentage of the inhibition of the inflammatory reaction was calculated with the following formula:

\[
\% \text{of inhibition} = \left(1 - \frac{\text{change of paw volume in the analyzed animal}}{\text{mean change of the paw volume in CAR group}}\right) \times 100.
\]

2.8. Isolation of Stomach and Collection of Gastric Juice. After euthanasia, the abdomen was opened and the stomach excised. The removed stomach was opened along greater curvature, and gastric content was rinsed with 5 mL of distilled water into a centrifuge tube. The obtained solution was centrifuged at 3000 rpm for 15 min. The supernatant was used for pH measurement. The cleaned stomach was preserved in 0.1 M phosphate-buffered saline (1:4 (w/v), pH 7.4) prior to macroscopic examination. Then, the stomach was fixed in 4% buffered formalin, embedded in paraffin, and cut into 4 μm thick slices, which were mounted on the glass slides and stained by the routine hematoxylin-eosin (H&E) method.

2.9. Measurement of the pH of Gastric Juice. The pH of gastric juice was measured with GLP 21 pH meter (Crisson Instruments SA, Barcelona, Spain) according to the manufacturer’s instruction.

2.10. Macro- and Microscopic Examination of Gastric Mucosa. The damage of the gastric mucosa was assessed in macro- and microscopic examination. The severity of macroscopically visible changes in the mucous membrane was evaluated using the J-scoring method, classifying the erosions as follows: no erosions = 0; 0-1 mm in diameter = 1; 1-2 mm = 2; and greater than 2 mm in diameter = 3. The sum of these measured areas in each animal was described as the gastric index [31]. Histopathological changes of all stomach specimens were examined in a blinded way by the experienced pathologist. The inflammation process and the damage of gastric mucosa were assessed independently. The severity of the inflammation was assessed using 0-3 scale (0: no inflammation, 1: mild inflammation, 2: moderate, and 3: severe inflammation). The severity of the damage of gastric mucosa was assessed using 0-3 scale (0: no damage, 1: superficial erosion, 2: submucous ulceration, and 3: ulceration in muscularis propria). The cumulative microscopic gastric index was defined as the sum of the inflammation and damage score.

2.11. Statistical Analysis. All experimental data are presented as the mean values ± standard deviation (SD). Statistical differences between studied parameters were analyzed using one-way analysis of variance (ANOVA) and NIR Fischer post hoc test. The comparison of the anti-inflammatory activity of the investigated doses of the tested fraction and indomethacin was performed with the multicriteria decision...
3. Results

3.1. Carrageenan Paw Oedema Test. Paw oedema in the experimental groups is shown in Figures 2 and 3. A significant increase in paw volume was observed in all groups that received subplantar injection of 1% carrageenan solution compared to the CON group 2 hours after the injection and sustained increase throughout the experiment. The enhancement in paw volume was lower in rats receiving indomethacin and the investigational fraction of alkaloids in both higher doses (5 mg/kg and 10 mg/kg) compared to an untreated positive control (CAR). The inhibitory effect of the lower dose (5 mg/kg) lasted shorter than the effect of the higher dose (10 mg/kg) of alkaloid fraction and indomethacin. The inhibition of the inflammatory reaction with indomethacin and the investigational fraction is presented in Figure 4. At the end of the experiment, the inhibitory effect of the highest dose of alkaloid fraction (10 mg/kg) did not differ significantly from the effect of indomethacin. Lower doses of the investigational fraction containing sanguinarine and chelerythrine (1 mg/kg and 5 mg/kg) inhibited the paw oedema to a lesser extent than indomethacin.

3.2. Assessment of TNFα, PGE2 and MMP-9. The enzyme-linked immunosorbent assay (ELISA) was used to assess the concentration of TNFα, PGE2, and MMP-9 in the homogenized subplantar tissue (Table 1). The injection of 1% carrageenan solution induced a potent production of TNFα, PGE2, and MMP-9, which was significantly inhibited with indomethacin 10 mg/kg and an investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine (1, 5, and 10 mg/kg, respectively). Data are presented as the mean ± SD.

3.3. Histopathological Assessment of Gastric Mucosa. To assess the gastric safety profile of the investigated compounds, macroscopic (Figure 5) and microscopic (Figure 6) examinations of the stomachs were performed. The presence and severity of macroscopically visible lesions (petechiae, hemorrhagic erosions) were scored as the indicators of ulcerogenic activity (cumulative macroscopic gastric index calculated as the sum of the measured pathological areas evaluated using the J-scoring method). Macroscopic examination of the gastric mucosa demonstrated that investigational fraction of *C. chinensis* in all assessed doses caused negligible mucosal lesions (Figures 5(a)–5(c) and Table 2, p > 0.05 vs. CON), whereas indomethacin given in the dose of 10 mg/kg caused significant gastric injuries (Figure 5(d) and Table 2, p < 0.0001 vs. CON). The microscopic assessment was in line with the values obtained macroscopically. The stomach tissue of rats pretreated with SC fraction as well as the control rats showed no significant histopathological changes (Figures 6(a)–6(c), 6(e), and 6(f) and 6(c), 6(e), and 6(f)) and showed negligible mucosal lesions (Figures 5(a)–5(c) and Table 2, p > 0.05 vs. CON), whereas indomethacin given in the dose of 10 mg/kg caused significant gastric injuries (Figure 5(d) and Table 2, p < 0.0001 vs. CON). The microscopic assessment was in line with the values obtained macroscopically. The stomach tissue of rats pretreated with SC fraction as well as the control rats showed no significant histopathological changes (Figures 6(a)–6(c), 6(e), and 6(f) and 6(c), 6(e), and 6(f)).

3.4. pH of Gastric Juice. A significant increase in pH of gastric juice was observed only in animals receiving a low dose (1 mg/kg) of investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine (Table 3).

3.5. Multicriteria Decision Analysis (MCDA). The results obtained in the carrageenan paw oedema test and ELISA performed for three doses of investigational alkaloid fraction and indomethacin were analyzed with MCDA to compare their anti-inflammatory activity. The results of MCDA (Figure 7) showed that the investigational fraction of *C. chinensis* acted dose-dependently and it exerted a relevantly lower anti-inflammatory effect than indomethacin 10 mg/kg only if given in the lowest dose of 1 mg/kg.

4. Discussion

The protective effect of sanguinarine-chelerythrine fraction of *C. chinensis* on acute inflammation in rat paw was investigated in the reported study. The study showed that the investigational fraction of *C. chinensis*, especially at the highest dose of 10 mg/kg, was capable of ameliorating carrageenan-induced rat paw oedema. Additionally, it reduced the carrageenan-induced secretion of PGE2, TNFα, and MMP-9 in the rat paw. The anti-inflammatory effect of the investigational fraction of *C. chinensis* was not associated with damage of gastric mucosa. To our knowledge, this was the first study to assess the anti-inflammatory potency of this fraction.
as well as to compare the effects of the SC fraction to the use of indomethacin in an acute inflammatory model.

One of the most commonly used tests to investigate the anti-inflammatory activity of experimental substances is the carrageenan rat paw oedema test. According to the guidelines, it is the first in vivo study, which should be performed after the in vitro tests of potentially anti-inflammatory plant-derived substances [32]. The carrageenan exerts a biphasic inflammatory effect. The first phase lasts 1 to 2.5 hours and is associated with the secretion of histamine and serotonin at the beginning followed by kinin release. In the second phase, lasting from 2.5 to 6 h, the

![Figure 3: Paw oedema in the experimental groups. CON: negative control group; CAR: positive control group; IND: group receiving indomethacin 10 mg/kg; SC₁, SC₅, and SC₁₀: groups receiving investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine (1, 5, and 10 mg/kg, respectively).](image)

![Figure 4: Inhibition of the paw oedema with indomethacin and investigational mixture 6 hours after the injection of 1% carrageenan solution. IND: group receiving indomethacin 10 mg/kg; SC₁, SC₅, and SC₁₀: groups receiving an investigational fraction of *C. chinensis* containing sanguinarine and chelerythrine (1, 5, and 10 mg/kg, respectively). Data are presented as the mean ± SD. *p < 0.05 vs. IND.](image)
inflammatory reaction is mediated mainly by excessive production of PGE$_2$ [33]. We observed significant paw oedema in the carrageenan-injected animals. The pretreatment with the alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine dose-dependently inhibited the second phase of carrageenan rat paw oedema in regard to the positive control group, suggesting the inhibition of PGE$_2$ release. Similar results were reported for the nonprotein fraction of *C. majus* extract that contains among others sanguinarine and chelerythrine [34] [34] [34]. In the reported study, six

![Figure 5: Macroscopic examination of the gastric mucosa. Experimental groups (n = 10): (a) group receiving investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine 1 mg/kg (SC$_1$); (b) group receiving investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine 5 mg/kg (SC$_5$); (c) group receiving investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine 10 mg/kg (SC$_{10}$); (d) group receiving indomethacin 10 mg/kg (IND); (e) positive control group (CAR); (f) negative control group (CON).](image-url)
hours after carrageen-administration, there was no significant difference in the inhibition of the paw oedema between the animals pretreated with indomethacin (the reference nonsteroidal anti-inflammatory drug) at the dose of 10 mg/kg and those that received the highest dose of the SC fraction (10 mg/kg). However, lower doses of an investigational fraction of *C. chinensis* were less potent than the reference drug.

In the present study, we found a significant increase in the levels of inflammatory mediators such as PGE$_2$ and TNF-$\alpha$ in the paw of carrageenan-administered animals. Prostaglandin E$_2$ is the most abundantly detected PG in various tissues and exerts versatile physiological and pathological actions via four EP1–4 receptor subtypes [35]. The role of PGE$_2$ in acute inflammation was initially associated with vasodilatation of vascular smooth muscle cells via the EP2/EP4 receptors; then, it was demonstrated that PGE$_2$ induces mast cell activation via EP3 and consequently enhances vascular permeability, contributing to PGE$_2$-induced acute inflammation [36]. Moreover, PGE$_2$ promotes T$_h$1-cell differentiation, T$_h$17-cell proliferation, and production of pro-inflammatory IL-22 from T$_h$22 cells in vitro via EP2 and EP4 and intensifies chronic inflammation mostly via the EP4 receptor [35]. In turn, TNF-$\alpha$ is believed to be a key pro-inflammatory cytokine involved in many pathological processes. TNF-$\alpha$ among others can stimulate the overexpression of COX-2 and subsequently the overproduction of PGE$_2$ [37]. The pretreatment with the alkaloid fraction of *C. chinensis* containing sanguinarine and
chelerythrine (in all investigated doses) decreased the level of PGE$_2$ in the paw after the carrageenan injection. This observation supports the hypothesis about the inhibition of PGE$_2$ synthesis, which can be at least partially attributed to the fact that chelerythrine and sanguinarine are reported to inhibit COX-2 in vitro ([W. [38, 39]). Apart from inhibiting the PGE$_2$ synthesis, the investigated alkaloid fraction decreased the production of TNFα. Several studies revealed that both alkaloids inhibit TNFα production in vitro [27, 40]. The results obtained by Meng et al. allow hypothesizing that the observed decrease of the TNFα concentration may be at least partially the consequence of the inhibitory influence of sanguinarine on TLR4/NFκB signalling pathway [8].

An important role in the progress of inflammation is played by zinc-dependent proteolytic enzymes—matrix metalloproteinases (MMP), including MMP-9. In pathological conditions, MMP-9 is upregulated during development and wound healing as well as during pathologies that involve inflammatory processes, including arthritis, diabetes, and cancer [41]. Certain dependence of COX expression on the presence of MMP-9 was also proved [42]. The imbalance between synthesis and degradation of MMP-9 contributes to inadequate tissue remodeling, which can consequently lead to the formation of ulcers in the mucosa [43]. It was shown that MMP-9 is one of the key enzymes involved in the degradation of intestinal tissue during an inflammatory disease of gastrointestinal tract, for example, Crohn’s disease (CD) and ulcerative colitis (UC) [44, 45]. In various experimental animal models, it was observed that the decrease of MMP-9 can result in the attenuation of inflammation in the intestine [46, 47]. In the present study, we observed the increased level of MMP-9 in the paw of carrageenan-administered animals. We have shown that the tested alkaloid fraction (as well as the positive control—indomethacin) significantly reduced the concentration of MMP-9 evoked by the inflammation induced by the carrageenan administration. Park et al. (S. Y. [48]) informed that sanguinarine inhibited TPA-induced MMP-9 mRNA and protein expression and MMP-9 enzymatic activity in a dose-dependent manner in the breast cancer model. Their examination also proved sanguinarine to reduce COX-2 and PGE$_2$ levels. Thus, the decrease in PGE$_2$ secretion reported in our study is in line with the above-mentioned report of Park et al. Depletion of MMP-9 expression in response to sanguinarine [49] and to chelerythrine has also been observed in other studies, where the inactivation of NFκB pathway was comitantly noted [25, 50, 51]. However, to our knowledge, up to date, no published reports have examined the impact of sanguinarine and chelerythrine on MMP-9 concentration in the classic inflammation model. Therefore, our findings are the first to suggest that this may

### Table 2: The impact of investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine on gastric mucosa. Indomethacin was used as a reference drug.

| Group | Macroscopic evaluation | Inflammation score (0-3) | Microscopic evaluation (H&E staining) | Cumulative microscopic gastric index (0-6) |
|-------|-------------------------|--------------------------|----------------------------------------|------------------------------------------|
|       | Gastric index           |                          |                                        |                                          |
| CON   | 0.50 ± 0.71***          | 0.571 ± 0.787***        | 0.143 ± 0.378***                      | 0.714 ± 1.113***                      |
| CAR   | 0.64 ± 1.08***          | 0.875 ± 0.354***        | 0.0 ± 0.0***                          | 0.875 ± 0.354***                      |
| IND   | 17.90 ± 8.56***         | 1.375 ± 0.518***        | 1.375 ± 0.518***                      | 2.750 ± 0.886***                      |
| SC$_1$| 0.0 ± 0.0***           | 0.375 ± 0.518***        | 0.125 ± 0.354***                      | 0.500 ± 0.756***                      |
| SC$_5$| 0.20 ± 0.42***         | 0.0 ± 0.0***            | 0.0 ± 0.0***                          | 0.0 ± 0.0***                          |
| SC$_{10}$| 0.0 ± 0.0***          | 0.250 ± 0.463***        | 0.0 ± 0.0***                          | 0.250 ± 0.463***                      |

CON: negative control group; CAR: positive control group; IND: group receiving indomethacin 10 mg/kg; SC$_1$, SC$_5$, and SC$_{10}$: groups receiving investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine (1, 5, and 10 mg/kg, respectively); H&E: hematoxylin-eosin. The severity of macroscopic gastric damage was evaluated using the J-scoring method, classifying the erosions as follows: 0: no erosion; 0-1 mm in diameter = 1; 1-2 mm = 2, greater than 2 mm in diameter = 3. The sum of these measured areas in each animal was described as the cumulative microscopic gastric index [31]. Microscopic evaluation assessed independently the inflammation process and the damage of gastric mucosa. The severity of the inflammation was assessed using 0-3 scale (0: no inflammation, 1: mild inflammation, 2: moderate, and 3: severe inflammation). The severity of the damage of gastric mucosa was assessed using 0-3 scale (0: no damage, 1: superficial erosion, 2: submucous ulceration, and 3: ulceration in muscularis propria). The cumulative microscopic gastric index was defined as the sum of the inflammation and damage score. Data are presented as the mean ± SD. ***p < 0.0001 vs. IND; ****p < 0.001 vs. CON.

### Table 3: The impact of investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine on pH of gastric juice. Indomethacin was used as a reference drug.

| Group | pH          |
|-------|-------------|
| CON   | 3.157 ± 0.457 |
| CAR   | 3.450 ± 0.761 |
| IND   | 3.234 ± 0.603 |
| SC$_1$| 4.282 ± 0.391** |
| SC$_5$| 3.274 ± 0.368 |
| SC$_{10}$| 3.464 ± 0.756 |

CON: negative control group; CAR: positive control group; IND: group receiving indomethacin 10 mg/kg; SC$_1$, SC$_5$, and SC$_{10}$: groups receiving investigational alkaloid fraction of *C. chinensis* containing sanguinarine and chelerythrine (1, 5, and 10 mg/kg, respectively). Data are presented as the mean ± SD. **p < 0.01 vs. IND.
be one of the mechanisms underlying the anti-inflammatory activity of both alkaloids.

It is worth noting that the anti-inflammatory activity of the C. chinensis fraction containing sanguinarine and chelerythrine was in multicriteria decision analysis comparable (groups SC₅ and SC₁₀) to that of indomethacin—a drug considered to be one of the strongest NSAIDs. However, the use of indomethacin in clinical practice is relatively limited, mostly to external use, e.g., in the form of an aerosol, mainly due to its ulcerogenic effect. In fact, indomethacin is commonly used orally for more serious conditions, such as arthritis. Taking into account the results of our study and recently published paper showing the effectiveness of sanguinarine in the treatment of neuropathic pain in animal models (P. [52]), we believe that investigated alkaloids may be a very valuable alternative to indomethacin, as their anti-inflammatory potency is comparable, but practically devoid of the acute ulcerogenic side effect of indomethacin (groups SC₃ and SC₁₀). Obviously, this evaluation should take into account the short duration of the experiment and the single one exposure of the tissue to the inflammatory factor. Due to the fact that indomethacin if applied is most commonly used in chronic inflammatory diseases, therefore, since the results of our study showed a certain therapeutic potential of the investigational fraction, further analyses are necessary for the complete comparison, including assays that would include multiple administration of the SC fraction and evaluation of its effects in the case of chronic exposure to inflammation. Sanguinarine and chelerythrine are characterized by a highly homologous structure; however, they differ in varied oxygen electron-donating substituents, which may potentially result in the diversified intensity of their anti-inflammatory effects [53]. An influential difference was also observed in the case of the anticancer effect. Many cancer cells develop a mechanism to foreclose programmed cell death through the overproduction of proteins that prevent apoptosis, e.g., Bcl-2 and Bcl-XL. Sanguinarine and chelerythrine may inhibit these proteins, thus leading to the death of cancer cells [54, 55]. Despite the structural homology between these two compounds, they appear to bind to various target regions of the Bcl-XL protein, and in both cases, the docking points are different than in other known Bcl-XL inhibitors (Y.-H. [56]).

The above results may suggest that there might be a potential additive synergism between these two compounds (due to e.g., various target points or intensity of particular molecular mechanisms), which could also be significant in terms of their anti-inflammatory activity, and the mixture of both alkaloids may be more effective than if substances were used individually. As our examination of the effects of the SC fraction have yielded promising results, further research, including comparison of the anti-inflammatory effects of the mixture and each of the compounds individually, is more than recommended.

In the present study, pretreatment with indomethacin was associated with a significant macroscopic and microscopic damage of gastric mucosa, being the sign of acute gastrototoxicity. However, we did not detect any acute ulcerogenic activity of investigated fraction. Macroscopic and microscopic assessments of the gastric mucosa revealed significant gastric damage in animals receiving indomethacin, whereas the stomachs in animals receiving tested alkaloids remained unaffected. The safer gastric profile of the chelerythrine-sanguinarine fraction suggests that these alkaloids inhibit COX-1 activity to a lesser extent than indomethacin. They may be selective or preferential COX-2 inhibitors, but further researches are needed to explore the actual influence of the investigated fraction of C. chinensis on COX-1 and COX-2 activity. Our results are in line with the observations reported by Khayyal et al. [57] that described ulceroprotective effect of lberostab containing among other alkaloids derived from C. majus. The gastroprotective effect of chelerythrine and sanguinarine in an animal model of ethanol-induced gastric ulcer was also reported by other authors (W. [38, 58]). Moreover, these alkaloids not only do not exhibit ulcerogenic effects but can also play even a prophylactic role. One of the most important risk factors for peptic ulcer disease is Helicobacter pylori infection. Mahady et al. proved that sanguinarine and chelerythrine can inhibit the growth of the bacteria, with a MIC₅₀ of 50.0 and 100.0 μg/mL, respectively [59].

Interestingly, usage of the assessed fraction of C. chinensis did not cause a significant alteration in pH of the gastric juice of the tested animals (groups receiving 5 and 10 mg/kg of investigational alkaloid fraction; only the SC₁ group showed a relevant increase compared to the IND group). This fact also may be of key importance in the context of the possible application of both compounds in clinical practice. Changes in the pH of gastric juice may have undesirable consequences, among other indigestions, decreased appetite, or dyspeptic disorders. Thus, the three observations made in our study: anti-inflammatory activity of tested alkaloids comparable to indomethacin, no pathological changes in the gastric mucosa, and no increase in gastric juice pH in the SC₅ and SC₁₀ groups, make up a very promising whole, potentially presenting higher doses of the sanguinarine-chelerythrine fraction of C. chinensis as an effective and at the same time relatively safe anti-inflammatory agent for therapeutic praxis.
5. Conclusion

Inflammatory diseases are a common therapeutic problem and nonsteroidal anti-inflammatory drugs are not deprived of side effects, of which ulcerogenic activity is one of the most frequent. In our study, we found that sanguinarine and chelerythrine fraction of natural origin possesses anti-inflammatory activity comparable to indomethacin, but without acute adverse effects on the gastric mucosa. We showed that these isoquinoline alkaloids isolated in a form of fraction from C. chinensis inhibited the second phase of carrageenan rat paw oedema in a dose-dependent manner, suggesting the inhibition of PGE2 release, decreased the production of TNFα, and reduced the concentration of MMP-9. The obtained results present the fraction of sanguinarine and chelerythrine as a promising candidate for further research on new anti-inflammatory and analgesic drugs characterized with a safer gastric profile compared to existing NSAIDs.

Data Availability

The data underlying this article will be shared upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was funded by Wroclaw Medical University (grant number SUB.A080.21.021 (B.N.)) and by the National Science Centre of Poland (NCN) Sonata 15 (grant number 2019/35/D/NZ7/00266) (research project “Biotic and abiotic stress elicitors as modulators of isoquinoline alkaloid profile towards specific antimicrobial properties of medicinal plants from the Papaveraceae” to S.Z.).

References

[1] WHO, Monographs on Selected Medicinal Plants VOLUME 3, WHO, 2007.
[2] J. Wang, L. Wang, G. H. Lou et al., “Coptidis rhizoma: a comprehensive review of its traditional uses, botany, phytochemistry, pharmacology and toxicology,” Pharmaceutical Biology, vol. 57, no. 1, pp. 193–225, 2019.
[3] E. Cui, X. Zhi, Y. Chen et al., “Coptis chinensis and Myrobalan (Terminalia chebula) can synergistically inhibit inflammatory response in vitro and in vivo,” Evidence-based Complementary and Alternative Medicine, vol. 2014, 8 pages, 2014.
[4] F.-C. Meng, W. Zheng-Feng, Z.-Q. Yin, L.-G. Lin, R. Wang, and Q.-W. Zhang, “Coptidis rhizoma and its main bioactive components: recent advances in chemical investigation, quality evaluation and pharmacological activity,” Chinese Medicine, vol. 13, no. 1, p. 13, 2018.
[5] S. M. Park, B.-G. Min, J. Y. Jung et al., “Combination of pelargonium sidoides and Coptis chinensis root inhibits nuclear factor kappa B-mediated inflammatory response in vitro and in vivo,” BMC Complementary and Alternative Medicine, vol. 18, no. 1, p. 20, 2018.
[6] Z. Yang, V. Grinchuk, S. P. Ip et al., “Anti-inflammatory activities of a Chinese herbal formula IBS-20 in vitro and in vivo,” Evidence-based Complementary and Alternative Medicine, vol. 2012, Article ID 491496, 12 pages, 2012.
[7] M. Gilca, L. Gaman, E. Panait, I. Stoian, and V. Atanasiu, “Chelidonium majus-an integrative review: traditional knowledge versus modern findings,” Complementary Medicine Research, vol. 17, pp. 241–248, 2010.
[8] Y.-y. Meng, Y. Liu, H. Zhe-fu et al., “Sanguinarine attenuates lipopolysaccharide-induced inflammation and apoptosis by inhibiting the TLR4/NF-KB pathway in H9c2 cardiomyocytes,” Current Medical Science, vol. 38, no. 2, pp. 204–211, 2018.
[9] S. Zielinska, A. Jezierska-Domaradzka, M. Wójciak-Kosior, I. Sowa, A. Junka, and A. M. Matkowski, “Greater Celandine’s ups and downs-21 centuries of medicinal uses of Chelidonium majus from the viewpoint of today’s pharmacology,” Frontiers in Pharmacology, vol. 9, p. 299, 2018.
[10] S. Zielinska, M. Wójciak-Kosior, M. Dziagwa-Becker et al., “The activity of isoquinoline alkaloids and extracts from Chelidonium majus against pathogenic bacteria and Candida Sp,” Toxins, vol. 11, no. 7, 2019.
[11] A. Croaker, G. King, J. Pyne, S. Anoopkumar-Dukie, and L. Liu, “anguinaria canadensis: traditional medicine, phytochemical composition, biological activities and current uses,” International Journal of Molecular Sciences, vol. 17, no. 9, article 1414, 2016.
[12] F. Zhang, J. Xie, G. Wang, G. Zhang, and H. Yang, “Anti-osteoporosis activity of Sanguinarine in preosteoblast MC3T3-E1 cells and an ovariectomized rat model,” Journal of Cellular Physiology, vol. 233, no. 6, pp. 4626–4633, 2018.
[13] H. Ming, H. W. C. Chien, Y. W. Cheng, and J. J. Kang, “Mechanisms underlying the induction of vasorelaxation in rat thoracic aorta by sanguinarine,” Japanese Journal of Pharmacology, vol. 85, no. 1, pp. 47–53, 2001.
[14] R. Singh, I. Mackraj, R. Naidoo, and P. Gathiram, “Sanguinarine downregulates AT1a gene expression in a hypertensive rat model,” Journal of Cardiovascular Pharmacology, vol. 48, no. 2, pp. 14–21, 2006.
[15] C. M. Hu, Y. W. Cheng, J. W. Liao, H. W. Cheng, and J. J. Kang, “Induction of contracture and extracellular Ca2+ influx in cardiac muscle by sanguinarine: a study on cardiotoxicity of sanguinarine,” Journal of Biomedical Science, vol. 12, no. 2, pp. 399–407, 2005.
[16] J.-H. Jeng, W. Hui-Lin, B.-R. Lin et al., “Antiplatelet effect of sanguinarine is correlated to calcium mobilization, thromboxane and cAMP production,” Atherosclerosis, vol. 191, no. 2, pp. 250–258, 2007.
[17] C. Fu, G. Guan, and H. Wang, “The anticancer effect of sanguinarine: a review,” Current Pharmaceutical Design, vol. 24, no. 24, pp. 2760–2764, 2018.
[18] H. Huang, J. Yao, K. Liu et al., “Sanguinarine has anthelmintic activity against the enteral and parental phases of _trichinella_ infection in experimentally infected mice,” Acta Tropica, vol. 201, no. January, article 105226, 2020.
[19] Q. Zhang, Y. Lyu, J. Huang et al., “Antibacterial activity and mechanism of Sanguinarine against Providencia rettgeri in vitro,” PeerJ, vol. 8, article e9543, 2020.
[20] M. Luo, Y. Peili, K. Ni et al., “Sanguinarine rapidly relaxes rat airway smooth muscle cells dependent on TAS2R signaling,”
[26] Y. Zhu, Y. Pan, G. Zhang et al., “K. P. et al., vol. 104, no. 1, pp. 15 of Pathology vol. 20, no. 18, p. 4367, 2019.

[27] H. Ma, S.-R. Chen, H. Chen, and H.-L. Pan, “In vitro anti-fungal activity and possible mechanisms of action of chelerythrine,” Pesticide Biochemistry and Physiology, vol. 164, pp. 140–148, 2020.

[28] E. Kolaczkowska, B. Plytycz, B. Arnold, H. Piccard, and M. E. Czerwińska, “Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 in hyper-inflammatory and immune diseases,” Immunology, vol. 128, no. 4, pp. 739–416, 2013.

[29] I. Siloși, M. V. Boldea, S. Radna, M. V. Boldea, and S. Stoica, “Matrix metalloproteinase-9 [MMP-9] as a marker of inflammatory bowel disease (IBD),” Romanian Journal of Morphology and Embryology, vol. 55, no. 4, pp. 1317–1324, 2014.

[30] C. J. Bailey, R. M. Hembry, A. Alexander, M. H. Irving, M. E. Grant, and C. A. Shuttleworth, “Distribution of the matrix metalloproteinases stromelysin, gelatinases A and B, and collag enase in Crohn’s disease and normal intestine,” Journal of Clinical Pathology, vol. 47, no. 2, pp. 113–116, 1994.

[31] M. J. Meijer, M. A. Mieremet-Ooms, A. M. van der Zon et al., “Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn’s disease phenotype,” Digestive and Liver Disease, vol. 39, no. 8, pp. 733–739, 2007.

[32] E. Kolaczkowska, “Metalloproteinase 9 [MMP-9] as a unique member of the matrix metalloproteinase family: role in infl uence of neutrophils and their apoptosis during inflammation,” Postepy Biologii Komórki, vol. 37, no. 2, pp. 471–499, 2010.

[33] A. Santana, C. Medina, M. C. Paz-Cabrera et al., “Attenuation of dextran sodium sulphate induced colitis in matrix metalloproteinase-9 deficient mice,” World Journal of Gastroenterology, vol. 12, no. 40, pp. 6464–6472, 2006.

[34] S. Y. Park, M. L. Jin, Y. H. Kim, S.-J. Lee, and G. Park, “Sanguinarine inhibits invasiveness and the MMP-9 and COX-2 expression in TPA-induced breast cancer cells by inducing HO-1 expression,” Oncology Reports, vol. 31, no. 1, pp. 497–504, 2014.
[49] Y. H. Choi, W. Y. Choi, S. H. Hong et al., “Anti-invasive activity of sanguinarine through modulation of tight junctions and matrix metalloproteinase activities in MDA-MB-231 human breast carcinoma cells,” Chemico-Biological Interactions, vol. 179, no. 2–3, pp. 185–191, 2009.

[50] Y. Ji, J. Liu, Z. Wang, and N. Liu, "Angiotensin II induces inflammatory response partly via toll-like receptor 4-dependent signaling pathway in vascular smooth muscle cells," Cellular Physiology and Biochemistry, vol. 23, no. 4–6, pp. 265–276, 2009.

[51] Z. Xie, M. Singh, and K. Singh, "Differential regulation of matrix metalloproteinase-2 and -9 expression and activity in adult rat cardiac fibroblasts in response to interleukin-1β," Journal of Biological Chemistry, vol. 279, no. 38, pp. 39513–39519, 2004.

[52] P. Li, Y.-X. Wang, G. Yang, Z.-C. Zheng, and Y. Chao, "Sanguinarine attenuates neuropathic pain in a rat model of chronic constriction injury," BioMed Research International, vol. 2021, Article ID 3689829, 8 pages, 2021.

[53] M. Colombo and E. Bosisio, "Pharmacological activities of Chelidonium majus L. (Papaveraceae)," Pharmacological Research, vol. 33, no. 2, pp. 127–134, 1996.

[54] P. H. Bernardo, K.-F. Wan, T. Sivaraman et al., "Structure-activity relationship studies of phenanthridine-based Bcl-XL inhibitors," Journal of Medicinal Chemistry, vol. 51, no. 21, pp. 6699–6710, 2008.

[55] Y. Dai and S. Grant, "Targeting multiple arms of the apoptotic regulatory machinery," Cancer Research, vol. 67, no. 7, pp. 2908–2911, 2007.

[56] Y.-H. Zhang, A. Bhunia, K. F. Wan et al., "Chelerythrine and Sanguinarine dock at distinct sites on BclXL that are not the classic BH3 binding cleft," Journal of Molecular Biology, vol. 364, no. 3, pp. 536–549, 2006.

[57] M. Khayyal, M. El-Ghazaly, S. Kenawy et al., "Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination," Arzneimittel-Forschung, vol. 51, no. 7, pp. 545–553, 2001.

[58] Q. Sun, W. Li, H. Li, X. Wang, Y. Wang, and X. Niu, "Preparation, characterization and anti-ulcer efficacy of Sanguinarine loaded solid lipid nanoparticles," Pharmacology, vol. 100, no. 1–2, pp. 14–24, 2017.

[59] G. B. Mahady, S. L. Pendland, A. Stoia, and L. R. Chadwick, "In vitro susceptibility of Helicobacter pylori to isoquinoline alkaloids from Sanguinaria canadensis and Hydrastis canadensis," Phytotherapy Research, vol. 17, no. 3, pp. 217–221, 2003.