Society of Medical Education & Research

2024, Volume 4, Issue 1, Page No: 95-103
ISSN: 3108-4826

Journal of Medical Sciences and Interdisciplinary Research

HPLC Analysis and Mechanistic Insights into Achillea odorata's Effects on Gastric Emptying and Intestinal Transit Slowdown

Andreas Soren Kristensen[1]*, Mikkel Jonas Pedersen[1]

[1]Department of Management, Copenhagen Business School, Copenhagen, Denmark.

***E-mail** ✉ a.kristensen.cbs@outlook.com

Abstract

Plants of the Achillea genus have traditionally been employed to manage digestive disorders. This study aimed to investigate the mechanisms underlying the effects of the decocted extract of Achillea odorata L. (ADE), particularly its influence on neurotransmitters regulating gastrointestinal motility, as well as its impact on intestinal transit (IT) and gastric emptying (GE). Mice were administered ADE at doses of 100, 200, or 400 mg·kg^{-1}, followed one hour later by a phenol red meal. In a separate set of experiments, rats received ADE (200 mg·kg^{-1}) along with pharmacological agents including atropine (3.45 mmol·kg^{-1}), L-Nitro-N-Arginine (L-NNA) (1.36 mmol·kg^{-1}), or indomethacin (5.58 mmol·kg^{-1}) to evaluate its effects on IT and GE. ADE significantly reduced GE and IT at all tested doses, with GE values of 45.62±2.69 percent, 42.92±4.91 percent, and 28.80±3.02 percent, and IT values of 57.87±3.97 percent, 48.72±2.01 percent, and 42.81±3.96 percent, respectively. The observed delay in GE and antimotility effects were mediated via cholinergic, nitric oxide, and cyclooxygenase pathways. High-performance liquid chromatography with photodiode array detection (HPLC–DAD) identified 12 phenolic acid compounds in ADE, with chlorogenic acid being the most abundant at 33.43±0.18 mg·g^{-1}. These findings indicate that components of A. odorata L. could offer therapeutic potential in managing gastrointestinal motility disorders, such as diarrhea.

Keywords: Mice, Achillea odorata L., Decocted extract, Chemical profile, Gastric emptying, Intestinal transit

Introduction

Throughout history, various cultures have relied on medicinal plants to address a wide range of human health issues using traditional approaches. Given that gastrointestinal conditions can significantly impair well-being and, in severe cases, lead to mortality worldwide, there is growing attention toward identifying new therapeutic agents capable of managing these conditions effectively. Recent studies have explored the use of traditional remedies for both treating and preventing disorders affecting the digestive tract [1, 2].

Access this article online https://smerpub.com/

Received: 27 February 2024; **Accepted:** 23 April 2024

How to cite this article: Kristensen AS, Pedersen MJ. HPLC Analysis and Mechanistic Insights into Achillea odorata's Effects on Gastric Emptying and Intestinal Transit Slowdown. J Med Sci Interdiscip Res. 2024;4(1):95-103. https://doi.org/10.51847/Xp0ZoQEk4p

The gastrointestinal system plays essential roles in supplying the body with necessary nutrients, including vitamins, minerals, water, proteins, carbohydrates, and electrolytes. The regulation of gastric emptying (GE) and intestinal transit (IT) involves a sophisticated interplay of numerous neurotransmitters and mediators [3]. Disruptions in these regulatory processes can result in various disorders, such as diarrhea and constipation. Many pathophysiological issues within the gastrointestinal tract are linked to abnormalities in motility [4].

Both synthetic drugs and natural remedies have been employed to manage metabolic and health disturbances arising from irregularities in gastric motility and acid secretion. The utilization of medicinal plants dates back over two thousand years, and in recent times, there has been heightened consumer preference for herbal products due to their perceived efficacy and cost-effectiveness [5]. Among approximately 141 different medicinal plants

documented, around 34% are particularly utilized for alleviating gastrointestinal problems [6].

In the Compositae family, the genus Achillea, comprising over 120 species, holds significant value. Various studies have documented properties of Achillea species, including anti-inflammatory [7], antibacterial [8], antihypertensive [9], and antitumor effects [10]. Limited evidence exists regarding its gastrointestinal benefits, such as antispasmodic [11], choleretic [12], and antiulcer activities, and it is frequently employed in folk medicine for digestive complaints [11].

A. odorata L., a plant widely found across the Mediterranean area [13], has been traditionally applied for its anti-inflammatory properties (e.g., in rheumatism and allergic rhinitis) as well as for managing digestive issues. Nevertheless, scientific validation of its influence on gastrointestinal motility and gastric emptying remains lacking.

Data on the impact of A. odorata L. on GE and intestinal motility (IM) are presently unavailable. Thus, this investigation sought to evaluate the possible influences of a decoction extract from A. odorata L. in a mouse model, along with elucidating its underlying mechanisms. The research focused on determining potential interactions with neurotransmitters involved in regulating gastrointestinal motility. Furthermore, a phytochemical analysis was performed to pinpoint the main bioactive constituents in this species and their contributions to effects on gastrointestinal motility.

Materials and Methods

Plant material collection and authentication

A. odorata L. specimens were gathered from the Jijel region in northeastern Algeria during June 2020. Authentication was carried out by a botanist (Prof. Amira S.) at the Laboratory of Phytotherapy Applied to Chronic Diseases, where a voucher specimen was deposited under the reference number 302 AO 16/06/20 Jij/SA. The harvested plant material was dried in air for about 10 days to facilitate preservation, then ground into powder using an electric mill (RIRIHONG Brand High-speed Multifunctional Grinder, Japan).

Animals

Swiss albino mice (25–30 g) were obtained from the Pasteur Institute in Kouba, Algiers. They were acclimatized for one week in standard laboratory conditions with regulated temperature and humidity, provided ad libitum access to food (ONAB, Algeria), water, and normal activity. Prior to experiments, mice were individually housed in cages with wide-mesh wire floors. Water was available freely until 60 minutes before testing, while food was withheld for 18–20 hours during fasting, with no solid feed provided in that period.

All animal procedures adhered to the European Union guidelines on animal experimentation (2007/526/EC). Ethical approval for the experimental protocol was granted by the Scientific Council of the Faculty of Natural and Life Sciences, University of Setif-1 (Algeria).

Methods

Preparation of the decoction extract

The aqueous decoction was prepared according to the procedure described by Mamache *et al*. [14]. Fifteen grams of the powdered plant were boiled in 500 mL of water for 10 minutes, followed by cooling. The resulting mixture was filtered through Whatman No. 2 paper, transferred to plates, and dried in an oven (MEMMERT UM 400, ref. P2209-1894, Germany) at 38°C [14]. The final extract was kept refrigerated at 4°C in opaque, airtight containers to avoid light-induced degradation.

HPLC-DAD analysis

Phenolic constituents were examined using high-performance liquid chromatography with diode-array detection (HPLC-DAD; Shimadzu 20 AT series, Kyoto, Japan) equipped with an Intersil-ODS-3 reversed-phase C18 column [15, 16]. The flow rate was maintained at 1.0 mL/min, with a 20 µL injection volume. Mobile phases A and B both consisted of 0.5% acetic acid solutions. The gradient elution profile included: 0% B at start, rising to 10% B (0–0.01 min), then to 20% B (0.01–5 min), 30% B (5–15 min), 50% B (15–25 min), 65% B (25–30 min), 75% B (30–40 min), 90% B (40–50 min), and finally returning to 10% B (50–55 min).

Detection occurred via a photodiode array detector at 280 nm. Identification of phenolic compounds relied on comparisons of UV spectra and retention times with authentic standards. Analyses were replicated three times for reliability. Quantification involved calibration curves constructed from standard solutions at concentrations of 0.0, 0.00782, 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5, and 1.0 ppm. Results were expressed as grams per gram of dry weight.

Gastric emptying and small intestinal transit in mice

Assessments of gastric emptying (GE) and small intestinal transit (IT) were performed according to the method described by Amira *et al*. [17]. The test meal consisted of 1.5% carboxymethyl cellulose (CMC) dissolved in water with 0.1% phenol red added as a non-absorbable marker. Mice received 0.3 mL of this meal orally. Twenty minutes later, the animals were euthanized for further examination.

Following euthanasia, a laparotomy was conducted to excise the stomach and small intestine, with ligation of the pylorus and cardia. Gastric contents were homogenized in 25 mL of 0.1 N NaOH. To precipitate proteins, 8 mL of the homogenate was mixed with 1 mL of 33% (w/v) trichloroacetic acid and allowed to stand at room temperature for one hour. Absorbance was measured at 560 nm using a spectrophotometer (Shimadzu UV-1800, Germany).

In each experimental series, four mice were euthanized immediately after administration of the test meal to establish baseline values representing 0% emptying. The percentage of gastric emptying over the 20-minute period was determined using the following formula (Eq. 1):

$$GE\ (\%) = (AUntreated\text{-}Atreated/AUntreated) \times 100 \quad (1)$$

After stomach removal, the small intestine was carefully dissected free from most mesenteric attachments, and its total length was measured. The leading edge of the phenol red marker was visualized by applying a drop of 0.1 N NaOH, and intestinal transit was expressed as the percentage of the total small intestine length traversed by the meal front.

To explore potential mechanisms, GE and IT were evaluated in mice pretreated with atropine (3.45×10^{-3} mmol·kg^{-1}), L-NNA (1.36 mmol·kg^{-1}), or indomethacin (5.58×10^{-2} mmol·kg^{-1}), compounds known to interfere with neural or inflammatory pathways involved in gastrointestinal motility regulation.

Statistical analysis

In vivo data were analyzed using GraphPad Prism version 7.00. Values are presented as mean ± standard error of the mean (SEM). Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test. A P-value < 0.05 was considered statistically significant.

Results and Discussion

Phytochemical analysis

The decoction of A. odorata L. (ADE) was rich in phenolic compounds, quantified in mg·g^{-1} of dry extract. The predominant constituents identified were chlorogenic acid, ellagic acid, and luteolin **(Table 1 and Figure 1)**. Specifically, chlorogenic acid was the most abundant (33.43 ± 0.18 mg·g^{-1}), followed by luteolin (16.58 ± 0.22 mg·g^{-1}) and ellagic acid (12.35 ± 0.14 mg·g^{-1}).

These findings align closely with prior reports on Achillea millefolium, where HPLC-DAD analysis detected seven key phenolics, including apigenin-7-O-glucoside, chlorogenic acid, vicenin-2, luteolin-7-O-glucoside, rutin, luteolin, and apigenin [18]. Chlorogenic acid emerged as a prominent compound across studies. Bobis *et al*. [19] similarly reported chlorogenic acid, rutin, and luteolin as major phenolics in the leaves of A. millefolium.

Furthermore, analysis of the aqueous extract from Achillea santolinoides revealed derivatives such as apigenin (apigenin-2"-O-pentosyl-8-C-glucoside and apigenin-O-glucuronide), luteolin-7-O-rutinoside, and dicaffeoylquinic acids (3,5- and 3,4-isomers).

As noted by Birru *et al*. [20], diarrhea and constipation represent opposing disruptions in intestinal secretion, absorption, and motility, often causing abdominal discomfort, bloating, altered bowel habits, and pain. Impaired gastric emptying and motility alterations can also trigger nausea and vomiting [21]. The current investigation therefore examined the effects of ADE on both intestinal transit and gastric emptying.

The Achillea genus is characterized by a broad spectrum of bioactive compounds—including phenolic acids, flavonoids, coumarins, terpenes, lignans, and essential oils—underlying its reported antioxidant, antiulcer, antibacterial, antispasmodic, immunosuppressive, antitumor, and antidiabetic properties [22, 23]. Various species are traditionally employed worldwide for relieving abdominal pain, flatulence, diarrhea, and wounds, as well as for diuretic effects [24]. The concentrations of these phytochemicals are particularly relevant to their influence on gastrointestinal function.

Mechanisms underlying ADE-induced delay in intestinal transit and gastric emptying

Figure 2 illustrates the dose-dependent impact of ADE on intestinal motility. Administration of ADE at 100, 200, and 400 mg·kg^{-1} significantly reduced intestinal transit to 45.62 ± 2.69%, 42.92 ± 4.91%, and 28.80 ±

3.02%, respectively, compared to the control value of 62.31 ± 2.34% (P ≤ 0.0001).

Table 1. Phenolic profile of the aqueous decoction extract from A. odorata L., determined using HPLC-DAD analysis (expressed in mg·g⁻¹)[a]

Phenolic compound	Content in A. odorata (mg·g⁻¹)	Retention time (min)
Protocatechuic acid	2.24 ± 0.02	8.75
Chlorogenic acid	33.43 ± 0.18	12.35
p-Hydroxybenzoic acid	1.80 ± 0.06	12.77
6,7-Dihydroxycoumarin	0.59 ± 0.03	14.10
Coumarin	7.95 ± 0.08	24.49
Rutin	6.12 ± 0.05	25.30
Ellagic acid	12.35 ± 0.14	26.11
Rosmarinic acid	3.50 ± 0.06	26.77
Myricetin	0.87 ± 0.02	27.35
Luteolin	16.58 ± 0.22	31.70
Kaempferol	4.10 ± 0.08	33.21
Apigenin	3.95 ± 0.03	33.77

The results show [a] Data represent means ± standard error of the mean from three independent determinations ($p < 0.05$). Compounds not detected are indicated by "-".

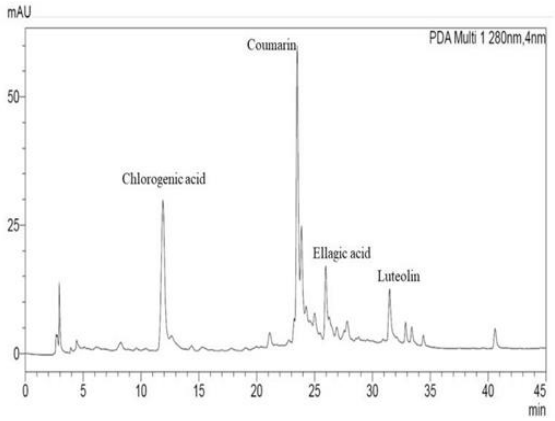

Figure 1. HPLC-DAD chromatogram of A. odorata L. decocted extract.

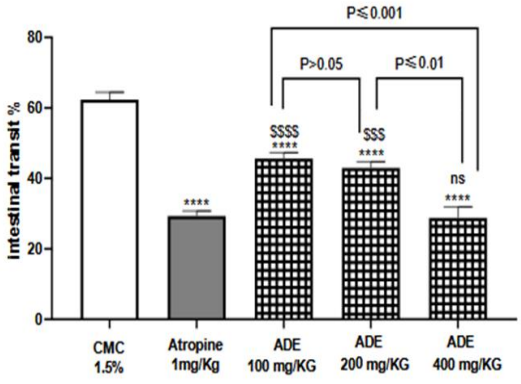

Figure 2. Effects of the ADE on intestinal transit in mice.

ADE; *A. odorata* L. decocted extract. Means ± SEM (n=9) is illustrated by bars. ****; P≤0.0001 vs vehicle (negative control). $$$; P≤0.001, $$$$; P≤0.0001 vs atropine (positive control). ns; not significant (P>0.05).

Additionally, as anticipated, antagonistic properties were observed following the application of the reference compound; atropine markedly lowered intestinal transit (IT) evaluation (29.29 ± 3.11%). In comparison to this atropine reference, solely the 400 mg·kg⁻¹ dosage exhibited no notable variation (P > 0.05).

The data indicate that oral delivery of A. odorata L. dichloromethane extract (ADE) exerted relaxant influences on gastric contents within the duodenum, thereby diminishing the overall gastric emptying (GE) rate, as illustrated in **Figure 3**. Prior treatment of mice with the extract at the tested doses (100, 200, or 400 mg·kg⁻¹) attenuated this process in a concentration-reliant fashion (80.11 ± 3.99%, 57.87 ± 3.97%, and 48.72 ± 2.01%), relative to the control group (42.81 ± 3.96%). Relative to the atropine positive control (44.26 ± 2.73%), ADE at 200 or 400 mg·kg⁻¹ showed no marked differences (P > 0.05).

To elucidate the mechanism underlying the extract's influence on IT and GE, ADE (200 mg·kg⁻¹) was given in distinct trials alongside various pharmacological agents. Administration of atropine (Atr) (3.45×10^{-3} mmol·kg⁻¹), L-NNA (1.36 mmol·kg⁻¹), or indomethacin (Indo) (5.58×10^{-2} mmol·kg⁻¹) caused a substantial decline (P ≤ 0.0001) in IT to 29.29%, 35.99%, and 44.27%, respectively **(Figure 4)**. These agents similarly lowered GE percentages to 42.82%, 33.78%, and 43.58% (P ≤ 0.0001) **(Figure 5)**.

Muscarinic receptor blockade via Atr combined with ADE resulted in a pronounced reduction (P ≤ 0.0001) in IT (27.93 ± 2.36%) versus the vehicle (1.5% CMC). This IT level with Atr was notably lower (P ≤ 0.0001) than that seen with ADE alone, yet showed no meaningful change (P > 0.05) relative to animals treated solely with Atr.

Co-administration of the nitric oxide synthase inhibitor L-NNA with ADE led to a marked decrease in IT (36.86 ± 2.87%, P ≤ 0.0001) compared to the vehicle, with no notable distinction from L-NNA alone (P > 0.05). Likewise, intraperitoneal indomethacin (prostaglandin synthesis inhibitor) alongside ADE significantly lowered

IT (41.62 ± 2.13%, $P \leq 0.0001$) versus the vehicle, while remaining similar to ADE alone. The IT percentage for ADE plus Indo was equivalent to Indo by itself.

A notable reduction occurred with atropine combined with ADE (200 $mg \cdot kg^{-1}$) relative to the vehicle ($P \leq 0.0001$) and to ADE solitary ($P \leq 0.01$), yielding a GE of 34.20%. No meaningful difference ($P > 0.05$) emerged between Atr + ADE and Atr alone.

Oral ADE delivery markedly attenuated GE (24.8%; $P > 0.05$) under NOS inhibition. Effects of ADE with and without L-NNA differed substantially (26.13%; $P \leq 0.01$). No evident distinction appeared between ADE impacts in vehicle-treated animals and those receiving only L-NNA (35.05%; $P > 0.05$). Furthermore, ADE combined with indomethacin showed no significant deviation from the indomethacin-only group (34.08 ± 1.13; $P > 0.05$).

Processes that either inhibit or promote gastric motility, pyloric function, and intestinal propulsion regulate GE and small bowel transit [24]. Findings from this investigation demonstrated that mice pretreated with ADE exhibited robust, dose-related impairments in intestinal propulsion and gastric evacuation compared to controls. The fundus served as an indicator of fundic activity in GE of a phenol red marker due to elevated intragastric pressure gradients from its sustained contractions [25]. Factors such as meal volume, caloric content, fluid intake, nutrient composition, drug exposure, particle size, health status, and psychological stress all influence gastric evacuation rates [26].

The extract's prolongation of IT could stem from suppression of muscular contractions and/or augmentation of relaxant mediators in intestinal smooth muscle, whereas its GE delay may arise from gastric muscle relaxation and/or pyloric sphincter tightening [27].

These observations align with prior work on the same genus by Niazmand and Khoshnood [28], which reported inhibitory actions on gastric evacuation in both baseline and vagally stimulated conditions. Such suppression might involve antagonism of intracellular calcium release in gastric smooth muscle or acetylcholine-mediated calcium influx. Similarly, Karamenderes *et al*. [29] found that Achellia nobilis extract antagonized acetylcholine, preventing acetylcholine-evoked contractions in rat duodenal smooth muscle. Aqueous extract of A. santolinoides L. similarly prolonged GE and IT in mice [30].

Polyphenols are known to induce relaxation in spontaneous contractions across various smooth muscles, including vascular [31], detrusor [32], and myometrial [33] tissues. Within the gut, polyphenols suppress smooth muscle contractility. The pronounced relaxant activity of ADE on gastrointestinal musculature may be attributed to its substantial chlorogenic acid content, consistent with Posłuszny *et al*. [34], who verified chlorogenic acid's spasmolytic properties against acetylcholine-induced contractions. Apigenin, also present in the extract, potently inhibits LTD4-triggered contractions [35]. Prior studies link this flavonoid's effects to calcium dynamics, with apigenin dose-dependently relaxing murine gastric smooth muscle primarily by blocking voltage-gated calcium channels [36]. Conversely, rosmarinic acid likely contributes to GE delay through muscarinic receptor antagonism in murine ileum [37]. Intraperitoneal delivery of flavonoids identified in the extract (apigenin, myricetin, rutin) reduced IT by 28–69% in mice [38].

Polyphenols further modulate IT, conferring antidiarrheal benefits. Atropine, a competitive muscarinic antagonist of acetylcholine, suppresses gastric evacuation and small intestinal motility [39]. Per Bahekar *et al*. [40], this likely involves M1 receptor blockade on parietal cells to reduce acid secretion, plus M3 inhibition on gastrointestinal smooth muscle to promote relaxation and diminish tone/amplitude [40, 41]. The extract's suppressive effects mirrored those of atropine closely, implicating full muscarinic blockade. Thus, muscarinic pathways appear central to ADE's inhibition of gastric evacuation and intestinal propulsion.

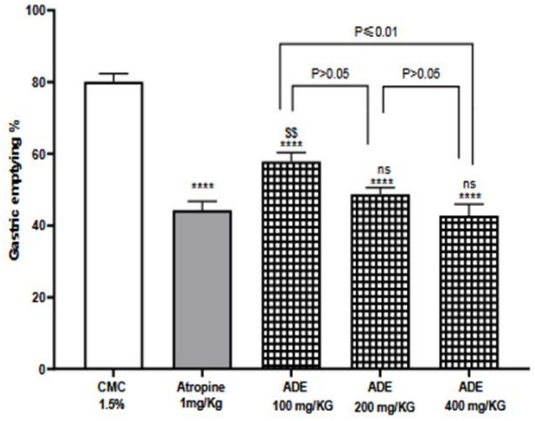

Figure 3. Influence of ADE on gastric emptying in mice. ADE: decoction extract of A. odorata L. Data are expressed as mean ± SEM (n=9 per group). **** indicates $P \leq 0.0001$ compared to vehicle (negative

control). $$ denotes P ≤ 0.01 relative to atropine (positive control). ns represents no statistical significance (P > 0.05).

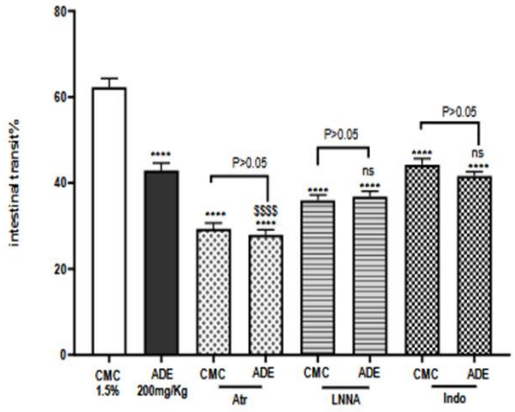

Figure 4. Effect of the ADE in the absence/presence of atropine, L-NNA or indomethacin on intestinal transit.

ADE; *A. odorata* L. decocted extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 vs vehicle as negative control. $$$$; P≤0.0001 in comparison between both effects of ADE in absence and in presence of atropine, L-NNA or indomethacin. ns; no significant difference (P>0.05).

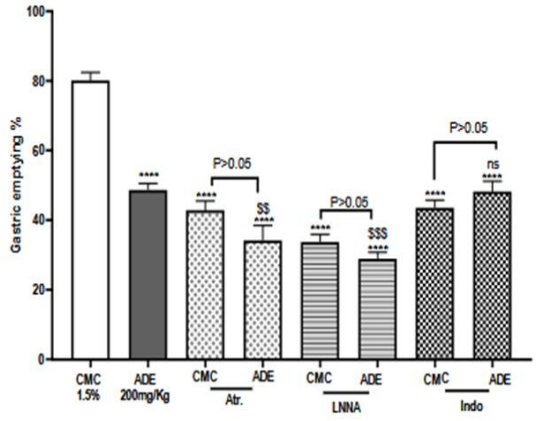

Figure 5. Effect of the ADE on gastric emptying in the presence or absence of atropine, L-NNA or indomethacin

ADE; *A. odorata* L. decocted extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 vs vehicle as negative control. $$; P≤0.01, $$$; P≤0.001 in comparison between both effects of ADE in the absence and in the presence of atropine, L-NNA or indomethacin. ns; no significant difference (P>0.05).

Nitric oxide synthase (NOS) catalyzes the synthesis of nitric oxide (NO) from L-arginine [42]. To date, two primary isoforms have been identified: a constitutive isoform (cNOS), which is Ca^{2+}/calmodulin-dependent and releases NO transiently in response to receptor activation, and an inducible isoform (iNOS), which operates independently of Ca^{2+} and sustains prolonged NO production following its expression. Certain analogs of L-arginine block both isoforms, while glucocorticoids specifically suppress iNOS induction [43]. NO acts as a non-adrenergic, non-cholinergic neurotransmitter and serves as a key mediator in the modulation of gastrointestinal motility by various agents. Pretreatment with L-NNA, a cNOS inhibitor, prolongs intestinal transit (IT) and delays gastric emptying (GE) [44]. These observations indicate that inhibitors of both constitutive and inducible NOS contribute to the ADE-mediated suppression of IT and GE, pointing to involvement of the nitric oxide pathway. Consistent with this, the present investigation showed that prior administration of L-NNA diminished the inhibitory actions of ADE on both IT and GE.

The cyclooxygenase (COX) enzyme transforms arachidonic acid (AA) into endogenous prostaglandins (PGs). Prostaglandins are recognized for their ability to induce contractions in gastrointestinal smooth muscle and exert diverse pharmacological influences on gut motility. For instance, prostaglandin $F_2\alpha$ ($PGF_2\alpha$) enhances the evacuation of liquids from the human stomach, whereas members of the PGE series relax the circular smooth muscle layer while contracting the longitudinal layer. In contrast, the PGF series promotes contraction in both layers [45]. Both PGE_2 and $PGF_2\alpha$ accelerate IT and can provoke diarrhea, unlike PGI_2 [46]. Indomethacin, as a COX inhibitor, blocks PG synthesis from AA and exhibits spasmolytic effects. In this work, pretreatment with indomethacin mitigated the ADE-induced delays in GE and prolongation of IT. Such results imply that endogenous prostaglandins act as intermediaries in the effects of ADE on GE and IT, thereby implicating the cyclooxygenase pathway.

Conclusion

The findings demonstrate that phenolic constituents in the decoction extract of Achellia odorata L. effectively impair gastric emptying and intestinal motility in mice via cholinergic, nitric oxide, and cyclooxygenase mechanisms. These outcomes support potential

antidiarrheal and/or antispasmodic activities of Achellia odorata L. Ongoing research aims to isolate the specific bioactive compounds accountable for these actions and to explore the potential contribution of additional pathways, including neural transmission and adrenergic receptors, to the observed inhibitions of GE and IT.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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