Original Research

Pharmacological Basis for the Antidiarrheal and Antispasmodic Effects of Cuminaldehyde in Experimental Animals: *In Silico*, *Ex Vivo* and *In Vivo* Studies

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Abstract

Background: Medicinal herbs are frequently used for the management of gastrointestinal disorders because they contain various compounds that can potentially amplify the intended therapeutic effects. Cuminaldehyde is a plant-based constituent found in oils derived from botanicals such as cumin, eucalyptus, myrrh, and cassia and is responsible for its health benefits. Despite the utilization of cuminaldehyde for several medicinal properties, there is currently insufficient scientific evidence to support its effectiveness in treating diarrhea. Hence, the present investigation was carried out to evaluate the antidiarrheal and antispasmodic efficacy of cuminaldehyde, with detailed pharmacodynamics explored. **Methods**: An *in vivo* antidiarrheal test was conducted in mice following the castor oil-induced diarrhea model, while an isolated small intestine obtained from rats was used to evaluate the detailed mechanism(s) of antispasmodic effects. **Results**: Cuminaldehyde, at 10 and 20 mg/kg, exhibited 60 and 80% protection in mice from episodic diarrhea compared to the saline control group, whereas this inhibitory effect was significantly reversed in the pretreated mice with glibenclamide, similar to cromakalim, an ATP-dependent K^+ channel opener. In the *ex vivo* experiments conducted in isolated rat tissues, cuminaldehyde reversed the glibenclamide-sensitive low K^+ (25 mM)-mediated contractions at significantly higher potency compared to its inhibitory effect against high K^+ (80 mM), thus showing predominant involvement of ATP-dependent K^+ activation followed by Ca^{++} channel inhibition. Cromakalim, a standard drug, selectively suppressed the glibenclamide-sensitive low K^+-induced contractions, whereas no relaxation was observed against high K^+, as expected. Verapamil, a Ca^{++} channel inhibitor, effectively suppressed both low and high K^+-induced contractions with similar potency, as anticipated. At higher concentrations, the inhibitory effect of cuminaldehyde against Ca^{++} channels was further confirmed when the preincubated ileum tissues with cuminaldehyde (3 and 10 mM) in Ca^{++} free medium shifted $CaCl_2$-mediated concentration-response curves (CRCs) towards the right with suppression of the maximum peaks, similar to verapamil, a standard Ca^{++} ion inhibitor. **Conclusions**: Present findings support the antidiarrheal and antispasmodic potential of cuminaldehyde, possibly by the predominant activation of ATP-dependent K^+ channels followed by voltage-gated Ca^{++} inhibition. However, further in-depth assays are recommended to know the precise mechanism and to elucidate additional unexplored mechanism(s) if involved.

Keywords: cuminaldehyde; antispasmodic; K^+ channel opener; Ca^{++} channel blocker; glibenclamide
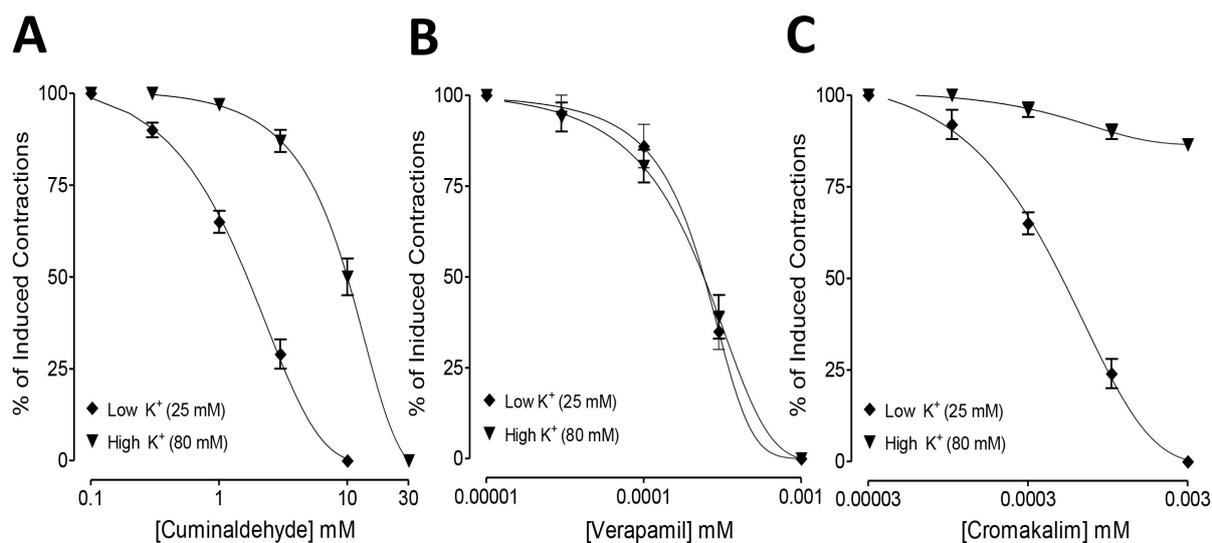
1. Introduction

Gastrointestinal disorder is characterized by dysfunctions in intestinal motility, which can manifest as abdominal pain, constipation, or diarrhea [1,2]. Gastrointestinal diseases, particularly constipation and diarrhea, affect 70% of the population worldwide [3]. Diarrhea is a significant cause of death in underdeveloped nations and is responsible for 1.5–2 million deaths in children under the age of five [4].

Medicinal herbs are commonly chosen for the treatment of gastrointestinal diseases due to the presence of several constituents that might enhance the desired effects or neutralize any potential negative effects [5,6]. As a result, they are generally considered safe for long-term usage. Herbal medicines show potential uses in the future because, for most plants, their chemical composition and pharmacological activities have yet to be thoroughly explored [7]. Plant derivative extract has also been used as a remedy, and many clinically valuable drugs have been derived from plant materials [8,9]. The seed husk of *Plantago ovata*, often known as psyllium husk, is a widely used plant-based medicine that both traditional healers and modern physicians favor as a complementary and alternative therapy [10]. Global interest in traditional medicine has surged, prompting initiatives to oversee and standardize herbal medications and traditional medicine [11]. Due to their perceived safety, natural products are increasingly favored over synthetic items, leading to a decline in the use of synthetic drugs [12].

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Fig. 1. Inhibitory concentration-response curves showing the comparison of (A) cuminaldehyde, (B) cromakalim, and (C) verapamil for the inhibitory effect against low K$^+$ (25 mM) and high K$^+$ (80 mM)-induced contractions in isolated rat ileum preparations. Values shown are mean ± SEM, n = 5.

Cumin seeds are sourced from the herb *Cuminum cyminum* Linn, which is a member of the Apiaceae family [13]. The seeds of cumin (*C. cyminum* L.) are popularly utilized as a spice due to their characteristic aroma. Additionally, they are frequently employed in traditional medicine to address various ailments such as chronic diarrhea, dyspepsia, acute gastritis, diabetes, and cancer [13]. The literature provides extensive evidence of the biological and medicinal effects of cumin, which are mostly attributed to its bioactive components, including terpenes, phenols, and flavonoids [14,15]. An investigation was conducted to evaluate the effect of cumin seed extract on diarrhea in albino rats induced with castor oil [16]. A natural monoterpenoid, cuminaldehyde, is a component of cumin oil and oils such as eucalyptus, myrrh, and cassia [17]. The health-promoting effects of cumin are attributed to cuminaldehyde [18], which has other functionalities such as anticancer [19], antidiabetic [20], neuroprotection [21], and anti-inflammation [22].

Although cuminaldehyde has been employed for several therapeutic purposes, there still needs to be more scientific evidence to substantiate its efficacy in the treatment of diarrhea and the detailed mechanism(s) involved. Therefore, the present study was designed to investigate the effectiveness with an exploration of the precise mechanism(s) involved in the potential gastrointestinal inhibitory effects of cuminaldehyde using *in vivo*, *ex vivo*, and *in silico* experiments.

2. Materials and Methods

2.1 Chemicals

Cuminaldehyde, carbamylcholine (CCh), loperamide, acetylcholine perchlorate (ACh), isoprenaline, verapamil, and papaverine were obtained from Sigma (St. Louis, MO, USA). Reagents (salts) to prepare a physiological buffer solution (Tyrode) were procured from Merck (Darmstadt, Germany). All chemicals used were of analytical grade, with the exception of castor oil, which was procured from a local pharmacy.

2.2 Animals

The Wistar albino rats (180–200 g) and Swiss albino mice (26–30 g) were obtained from the Animal Care Unit at the College of Pharmacy, Prince Sattam bin Abdulaziz University (PSAU), Saudi Arabia. They were kept at an optimal temperature of 22 ± 1 °C, with a relative humidity of 55 ± 5%, and were exposed to equal periods of light and darkness. All animals were given conventional pellet food and unlimited access to water. Stringent precautions were observed, and all studies (*in vivo* and *ex vivo*) adhered to the directions outlined in the National Research Council (NRC) [23]. The project has obtained approval from the Bio-Ethical Research Committee (BERC) at PSAU, with the reference number BERC-004-12-19.

2.3 In Vivo Antidiarrheal Study

A previously reported method was followed to test the possible antidiarrheal effect of cuminaldehyde [24]. A total of thirty-five mice were divided randomly into groups, ensuring that each group consisted of five mice. After fasting for twenty-four hours, the mice in the first group were given a saline solution (10 mL/kg) through oral gavage, and they were labeled as the negative control group. Following the pilot screening to determine the effective dose, the second and third groups (test groups) were subjected to two doses of cuminaldehyde (10 and 20 mg/kg). The mice in

Table 1. Effect of cuminaldehyde and cromakalim in the absence and presence of glibenclamide (3 mg/kg) on castor oil (10 mL/kg)-induced diarrhea in mice.

Treatment (p.o.)	Dose (mg/kg)	No. of mice with diarrhea (out of 5)	% protection
Saline + Castor oil (mL/kg)	10	5	0
Cuminaldehyde + Castor oil	10 + 10	2*	60
Cuminaldehyde + Castor oil	20 + 10	1*	80
Cromakalim + Castor oil	10 + 10	0**	100
Cuminaldehyde + Glibenclamide + Castor oil	10 + 3 + 10	4#	20
Cuminaldehyde + Glibenclamide + Castor oil	20 + 3 + 10	2	60
Cromakalim + Glibenclamide + Castor oil	10 + 3 + 10	4#	20

*$p < 0.05$ and **$p < 0.01$, compared to castor oil group, while # compared to without and with glibenclamide group. Chi-square test.
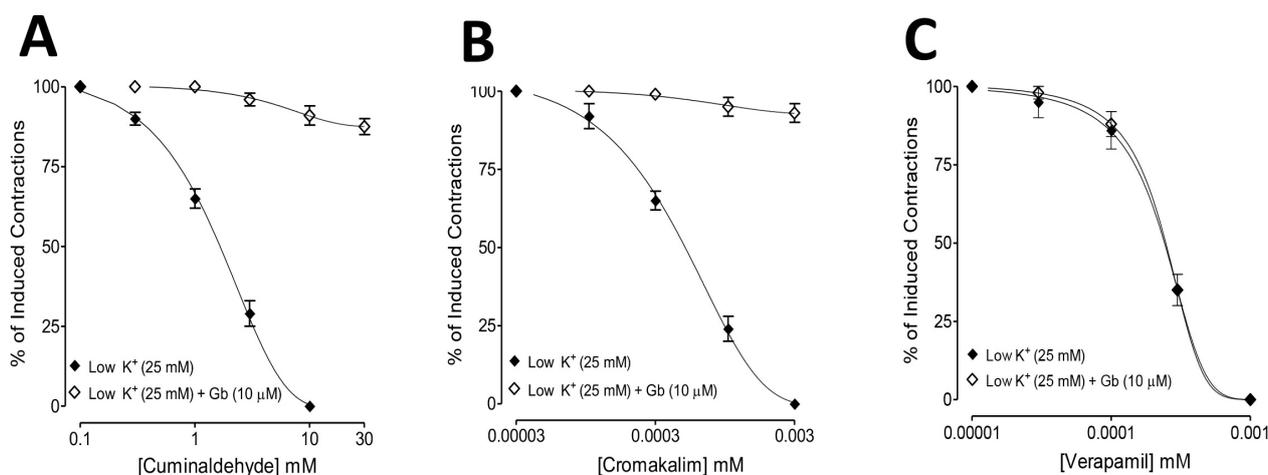

Fig. 2. Comparisons of the inhibitory concentration-response curves of (A) cuminaldehyde, (B) cromakalim, and (C) verapamil against low K$^+$ in the absence and presence of glibenclamide (Gb, 10 µM) in isolated rat ileum preparations. Values shown are mean $\pm$ SEM, n = 5.

the fourth group were exposed to cromakalim (10 mg/kg). The mice in the fifth, sixth, and seventh groups were given a pretreatment of glibenclamide (3 mg/kg) one hour before receiving cuminaldehyde or cromakalim. One hour after administration, all mice were orally administered castor oil (10 mL/kg) using a 1 mL syringe. Each mouse was then placed in an individual cage with a blotting sheet on the floor. After 4 hours, each cage blotting sheet was examined for the presence or absence of characteristic diarrheal droppings by a blind observer. The presence of protection was observed when no instances of diarrhea were detected, as previously documented by Jebunnessa *et al*. [25].

2.4 Ex Vivo Experiments on Isolated Rat Ileum

Prior to conducting *ex vivo* studies, rats underwent a 24-hour fasting period. Subsequently, their cervical dislocation was carried out under mild anesthesia. The ileum tissue was isolated using a previously described procedure [26]. After being isolated, specific sections of ileum tissues measuring 2–3 cm in length were thoroughly cleansed to remove nearby tissues and fecal matter. These cleaned tissues were then placed in an emkaBath apparatus (Paris, France) and connected to a transducer and IOX software (version 2.9.10.6, emka technologies, SAS, Paris, France). The tissue baths were filled with 20 mL of fresh tyrode solution (pH 7.4) and gassed with carbogen. The temperature was adjusted to 37 °C. A force of 1 gram was exerted by turning the transducer knob in a clockwise direction, and the tissues were allowed to stabilize for 30 minutes while being exposed to numerous doses of acetylcholine (0.3 µM). Following stabilization, sustained contractions were induced using low (25 mM) and high (80 mM) K$^+$ final bath concentrations (FBC). Additionally, cuminaldehyde was gradually introduced into the bath solution at concentrations ranging from 0.1 to 30 mM (FBC). The inhibitory effects of cuminaldehyde against K$^+$ (25 mM) and K$^+$ (80 mM)-mediated contractions have different meanings regarding the pharmacodynamics, including K$^+$ channel activation and/or voltage-gated Ca^{++} channel blockade. Godfraind *et al*. [27] found that high concentrations of K$^+$ (>30 mM) cause depolarization in various smooth muscles by activating Ca^{++} channels of the L-type. This leads to sustained
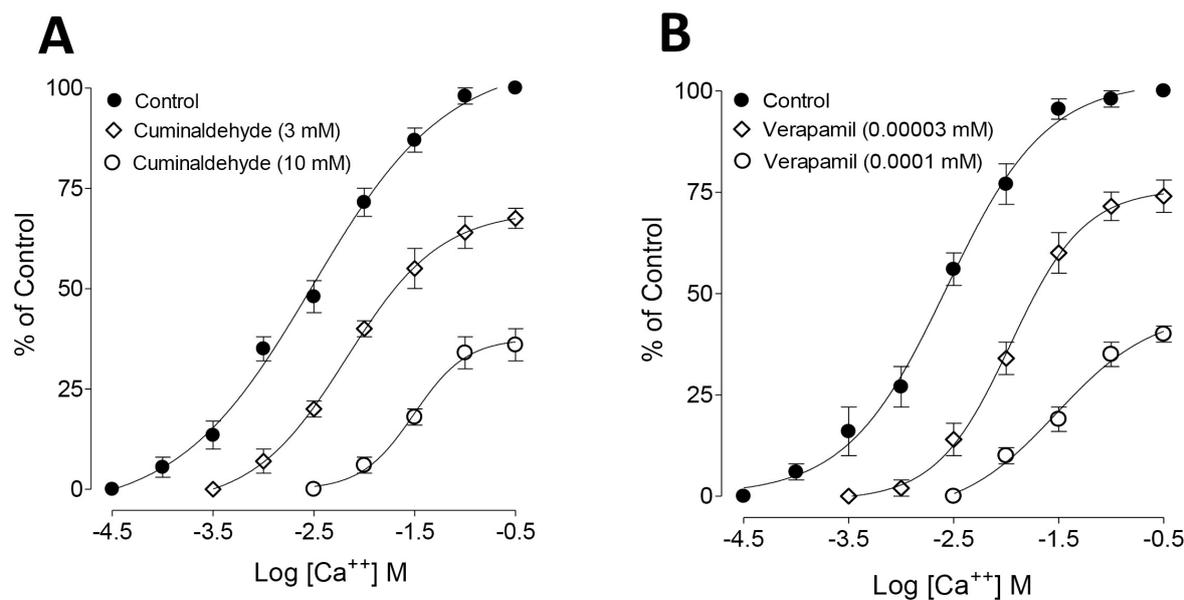
Fig. 3. Concentration-response curves of Ca^{++} in the absence and presence of the increasing concentrations of (A) cuminaldehyde and (B) verapamil in isolated rat ileum preparations. Values shown are mean $\pm$ SEM, n = 5.

contractions. Therefore, any substance that specifically and effectively inhibits the excitations mediated by low levels of K^+ is considered a K^+ channel activator. These types of assays are capable of distinguishing K^+ channel activators from Ca^{++} antagonists [28,29]. To know the possible involvement of the subtype, ATP-dependent K^+ channels, the inhibitory CRC of cuminaldehyde against low K^+ was reconstructed in the presence of glibenclamide, a known ATP-dependent potassium channel antagonist [30]. Parallel experiments were run with a positive control drug, cromakalim, a selective opener of ATP-dependent K^+ channels [31].

2.5 Ca^{++} Inhibitory Confirmation

After examining the relaxation effect of cuminaldehyde on high levels of K^+, further confirmation of calcium channel blocking (CCB) was achieved by incubating ileum tissues in a calcium-free Tyrode's solution with ethylenediaminetetraacetate (EDTA) (0.1 mM) for 45 minutes to remove calcium ions. In order to further reduce the levels of Ca^{++} within the cells, a solution without Ca^{++} was replaced with another solution known as K^+-rich and Ca^{++}-free Tyrode's solution. After incubating the tissue in this solution for 45 minutes, $CaCl_2$ CRCs were achieved in the absence and presence of increasing concentrations of cuminaldehyde. The results were then compared to the CRCs of a conventional CCB drug, verapamil [28].

2.6 Molecular Docking Studies

In order to get a molecular understanding of the predominant involvement of potassium channel activation by cuminaldehyde observed in the *ex vivo* assays, *in silico*

molecular modeling studies were performed on three distinct isoforms of ATP-dependent potassium channels with Protein Data Bank (PDB) IDs 7MJP, 7S61, and 6C3O. The protein structures of the receptors under consideration for study were obtained from the RCSB Protein Data Bank (RCSB PDB) website (https://www.rcsb.org/). The co-crystalline ligands of each receptor were isolated and redocked to validate the docking process. As indicated in our prior study work [32], Autodock Vina [33] was utilized for docking. The interaction between the docking confirmations and potassium channel receptors was observed using the Biovia Solutions Discovery Studio 4 programs [34]. The downloaded proteins were treated to remove water molecules, insert polar hydrogens, add Coleman charges, and store them in PDBQT format for docking. The structures of cuminaldehyde and cromakalim were retrieved in Stromal cell-derived factor (SDF) format from PubChem. In the next step, they were converted from SDF to PDB format and then written in the appropriate PDPQT format.

2.7 ADMET Studies

A chemical's ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics are critical for developing it as a lead medicine in the drug discovery cascade. The ADMET characteristics of cuminaldehyde were determined using the pkCSM software (https://biosig.lab.uq.edu.au/pkcsm/prediction), a web-based application [35]. Because this software allows smiles, the smiles were copied from PubChem and then sent to pkCSM for comprehensive profile prediction of the cuminaldehyde molecule.
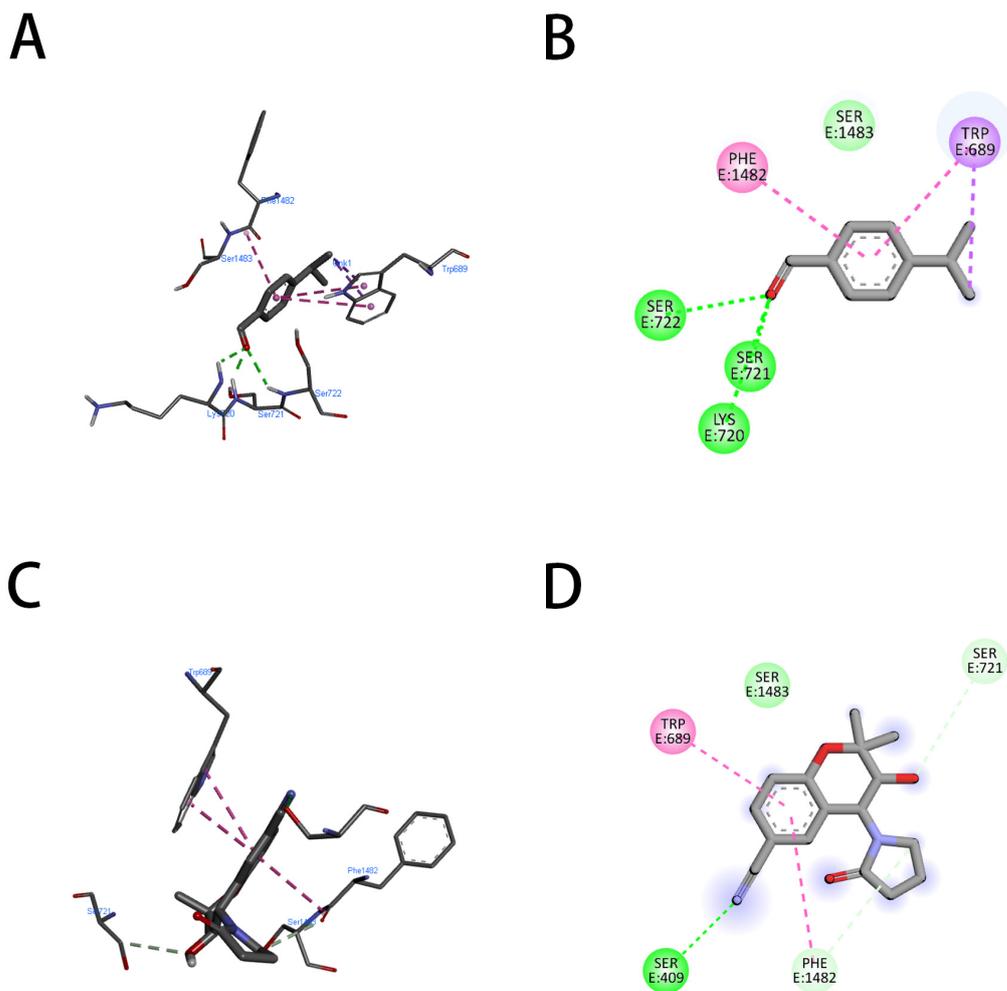
Fig. 4. Representation of the molecular interaction of cuminaldehyde (A,B) and cromakalim (C,D) with KATP (7S61). (A,C) 2-Dimensional and (B,D) 3-Dimensional.

2.8 Statistics

The results were provided as the mean $\pm$ SEM and the number of experiments conducted is denoted by "n". The median effective concentration (EC_{50}) values were evaluated with 95% confidence intervals (CI). The statistical analysis involved the use of Student's t-test or two-way ANOVA, followed by Bonferroni's post-test, to compare concentration-response curves (CRCs) with a control group. The efficacy of diarrhea protection was assessed by a statistical analysis, namely by comparing all groups to the saline control group using the Chi-square (χ^2) test. A p-value less than 0.05 is regarded as statistically significant. The regression analysis of CRCs was performed using GraphPad Prism (version 4, GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1 Antidiarrheal Effect

In mice, cuminaldehyde exhibited dose-dependent protection from diarrhea evoked by castor oil, while the saline group did not produce any protection. Preincubated mice with cuminaldehyde (10 and 20 mg/kg) exhibited 60% and 80% protection, whereas cromakalim (positive control group) produced 100% protection from diarrhea at 10 mg/kg. Glibenclamide (3 mg/kg) preincubation suppressed the antidiarrheal actions of cuminaldehyde and cromakalim, as detailed in Table 1.
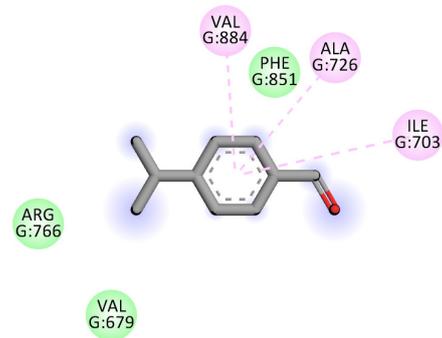
3.2 Ex Vivo Antispasmodic Effects

When checked against low and high K^+-mediated excitations, cuminaldehyde (Fig. 1A) suppressed the low K^+-mediated excitations with significantly higher potency (p
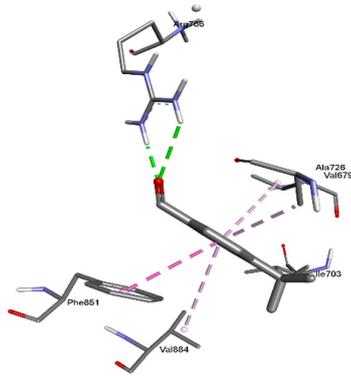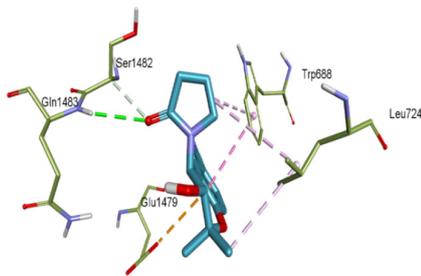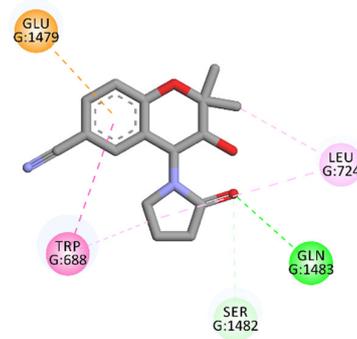
Fig. 5. Representation of the molecular interaction of cuminaldehyde (A,B) and cromakalim (C,D) with KATP (6C3O). (A,C) 2-Dimensional and (B,D) 3-Dimensional.

< 0.05) with EC_{50} values of 3.14 mM (2.78–3.92, 95% CI, n = 5) while low potency was observed in its inhibitory effect against the high K^+-mediated contractions [13.56 mM (12.20–14.42, 95% CI, n = 5)]. Cromakalim also selectively suppressed the low K^+-mediated excitations (Fig. 1B) with an EC_{50} value of 0.0008 mM (0.0002–0.0010, 95% CI, n = 5) while slightly suppressed the high K^+-mediated excitations with the highest % inhibition of (13.5 ± 1.5) whereas verapamil suppressed both types of low K^+ and high K^+-mediated excitations with comparable EC_{50} values of 0.00028 mM (0.00018–0.00045, 95% CI, n = 5) and 0.00024 μM (0.00017–0.00035) completely, respectively (Fig. 1C).

The inhibitory actions of cuminaldehyde and cromakalim against low K^+ were markedly reversed when the segments were preincubated with glibenclamide (Gb), re-sulting in 12.5 ± 2.5 and $7.0 \pm 3.0\%$ suppression, respectively (Fig. 2A,B), whereas verapamil was completely insensitive to Gb (Fig. 2C).

3.3 Calcium Channel Blocking (CCB)-Like Effect

To further confirm the Ca^{++} inhibitory effect, cuminaldehyde preincubated ileum tissues shifted the Ca^{++} CRCs curves at 3 and 10 mM towards the right, similar to that caused by verapamil at concentrations of 0.00003 and 0.0001 mM (Fig. 3A,B).

3.4 Molecular Docking Analysis

ATP-dependent potassium channel receptors are complex macromolecules consisting of sulfonylurea receptor (SUR) and inward rectifier Kir subunits, and they have been reported in RCSb in propeller and quatrefoil forms.
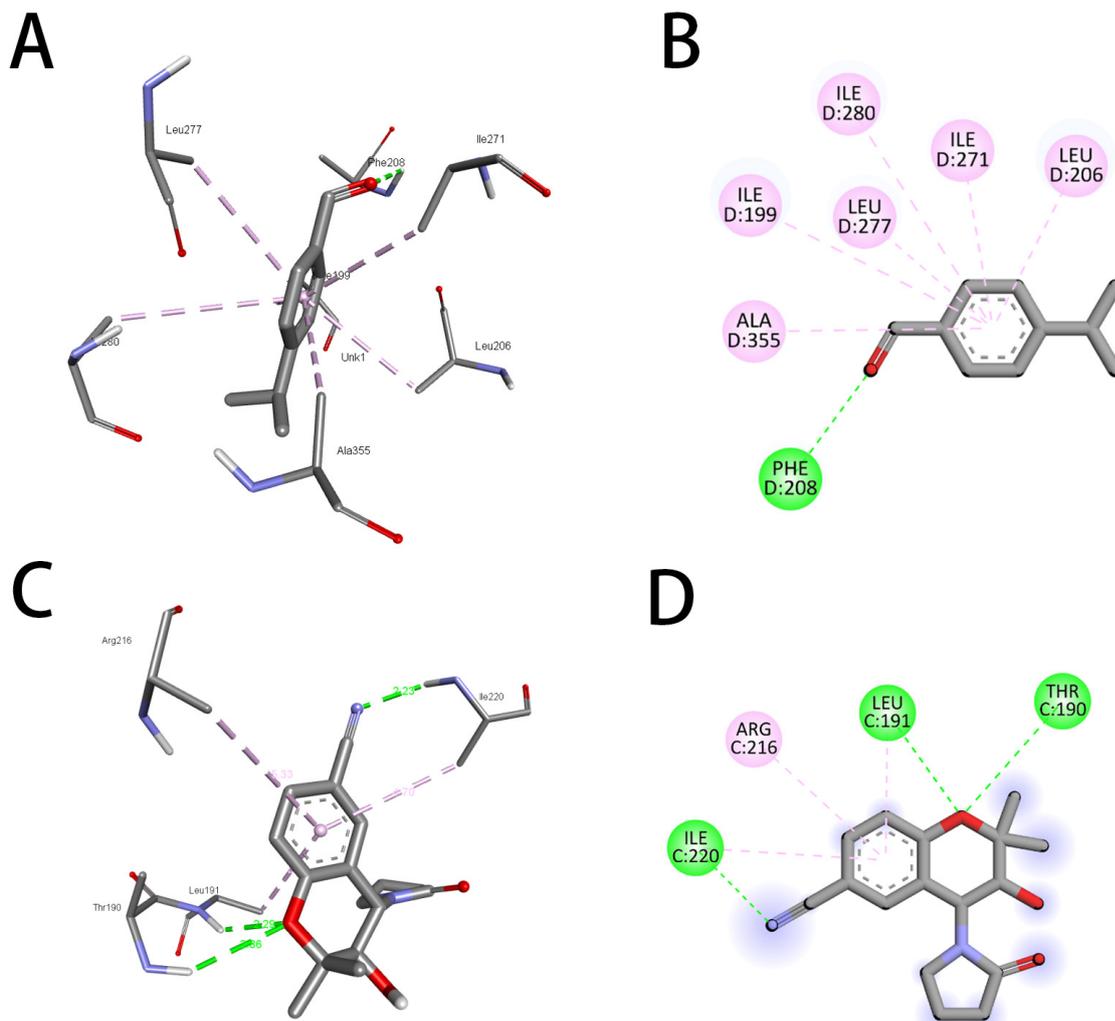
Fig. 6. Representation of the molecular interaction of cuminaldehyde (A,B) and cromakalim (C,D) with KATP (7MJP). (A,C) 2-Dimensional and (B,D) 3-Dimensional.

The pore opening in Kir is linked to coordinated structural changes in the ATP-binding site and the channel gate. The first chosen protein, 7S61, when subjected to molecular docking with cuminaldehyde and cromakalim, exhibited binding energies of –5.8 and –6.8 kcal/mol, respectively. For further confirmation, another human KATP (ATP-sensitive potassium channel) bound to ATP and ADP in quatrefoil form was downloaded and docked with the same two compounds considered in the study. The binding energies exhibited by cuminaldehyde and cromakalim this time were –6.8 and –7.8 kcal/mol, respectively. For further legitimization of this study, another ATP-dependent potassium channel, 7MJP, was considered for the docking study of the molecules in question, and they exhibited binding energies of –5.0 and –6.9 kcal per mole. The pattern of binding energy among all three receptors was found to be more or less similar. Cuminaldehyde and cro-

makalim have exhibited various forms of molecular interactions with the KATP proteins, as shown in Table 2 (Conventional H-Bond, Vander Waal, pi-pi stacking, Amide-pi stacking, Sigma-Pi stacking). Cuminaldehyde exhibited three conventional hydrogen bond interactions with the protein 7S61, while cromakalim exhibited only one. The hydrogen bond distance is also considered a crucial parameter for strong binding between ligand and receptor, the lesser the stronger. Cuminaldehyde showed shorter H-bond distances with *7S61* {a-1.91 (Ser721), b-2.32 (Lys720), c-2.48 (Ser722)}, on the other hand, cromakalim had longer {2.67 (Ser409)}. As shown in Fig. 4A–D, cuminaldehyde showed interactions with 6 residues (Trp-E689, Ser-E1483, Phe-E1482, Ser-E722, Ser-E721, Lys-E720), and cromakalim showed interactions with only 5 residues (Trp-E689, Ser-E1483, Phe-E1482, Ser-E409, Ser-E721). The KATP protein *6C3O*, when overviewed for such interaction, cumi-

Table 2. Molecular binding insights of cuminaldehyde and cromakalim with ATP-dependent potassium channel isoforms.

Receptor	Ligands	Binding Energy	Type of Interactions	H-Bond Distance	Residues
7S61	Cuminaldehyde	−5.8	Conventional H-Bond, Vander Waal, pi-pi stacking, Amide-pi stacking, Sigma-Pi stacking	a-1.91 (Ser721), b-2.32 (Lys720), c-2.48 (Ser722)	Trp-E689, Ser-E1483, Phe-E1482, Ser-E722, Ser-E721, Lys-E720
	Cromakalim	−6.8	Conventional H-Bond, Vander Waal, carbon H-Bond, pi-pi stacking, Sigma-Pi stacking	a-2.67 (Ser 409)	Trp-E689, Ser-E1483, Phe-E1482, Ser-E409, Ser-E721
6C3O	Cuminaldehyde	−6.8	Vander Waal, Sigma-Pi stacking	a-2.07 & 2.93 (Arg: 766)	Val679, Ile703, Ala726, Arg766, Phe851, Val784
	Cromakalim	−7.5	Conventional H-Bond, carbon H-Bond, pi-pi stacking, Anion-Pi stacking	a-2.99 (Gly1483)	Trp688, Glu1479, Ser1482, Gln1483
7MJP	Cuminaldehyde	−5	Conventional H-Bond, Vander Waal, Sigma-Pi stacking	a-2.69 (Phe208)	Ile199, Leu206, Ile271, Leu277, Ile280, Phe208, Ala355
	Cromakalim	−6.9	Conventional H-Bond, Sigma-Pi stacking	a-2.23 (Ile220), b-2.86 (Thr190), c-2.29 (Leu191)	Thr190, Leu191, Arg216, Ile220

naldehyde exhibited interactions (Vander Waal, Sigma-Pi stacking) with 6 residues (Val679, Ile703, Ala726, Arg766, Phe851, Val784), while cromakalim showed interactions (Conventional H-Bond, carbon H-Bond, pi-pi stacking, Anion-Pi stacking) with 4 residues (Trp688, Glu1479, Ser1482, Gln1483), as shown in Fig. 5A–D. Interestingly, cuminaldehyde made two Vander Waal interactions with bond distances of 2.07 and 2.93 with Arg766. At the same time, cromakalim exhibited a conventional H-bond but with a larger distance of 2.99 with Gly1483. Likewise, when the KATP protein 7MJP was overviewed cuminaldehyde exhibited interaction (Conventional H-Bond, Vander Waal, Sigma-Pi stacking) with 7 residues (Ile199, Leu206, Ile271, Leu277, Ile280, Phe208, Ala355) while cromakalim shown interaction (Conventional H-Bond and Sigma-Pi stacking) with 4 residues (Thr190, Leu191, Arg216, Ile220) as shown in Fig. 6.

3.5 ADMET Profiling

As shown in Table 3, cuminaldehyde has exhibited acceptable ADMET profiling. ADMET profiling is a must for establishing the drug-like properties of any molecule. The ADMET properties of cuminaldehyde were calculated by PKCSM software and exhibited an intestinal absorption close to 95%, and volume distribution (steady state) was found to be 0.324 (log L/kg). The log BB quotient for blood-brain barrier permeability was found to be 0.438, showing good BBB permeability. Cuminaldehyde does not inhibit any family of CYP450 enzymes except CYP1A2 and has a safe toxicity profile, except for being sensitive to the skin.

4. Discussion

The current study tested cuminaldehyde, a plant-based derived compound, for its antidiarrheal and antispasmodic actions in the *in vivo*, *ex vivo* and *in silico* models. Diarrhea is one of the leading diseases of the gastrointestinal system worldwide [36]. The castor oil-evoked diarrhea model, considered one of the well-accepted models for antidiarrheal screening drugs [37,38], was followed. Cuminaldehyde exhibited a dose-dependent protection, similar to cromakalim, a K^+ channel activator [31,39]. To check if the antidiarrheal action of the cuminaldehyde was mediated via the activation of ATP-dependent K^+ channels, animals were preincubated with Gb, a selective antagonist of ATP-activated K^+ channels [30,40], which significantly reversed the previously observed protective action of cuminaldehyde against diarrhea, similar to cromakalim, indicating the involvement of ATP-sensitive K^+ channels. Activators of the K^+ channel are also reported to possess antidiarrheal potential [41]. The dose for diarrhea protection was based on the previously reported acute toxicity study that revealed the safety of cuminaldehyde up to the maximum tested dose of 400 mg/kg [42]. Hence, our tested dose for diarrheal protection is 40 (10 mg/kg) and 20 (20 mg/kg) times less than the maximum safe dose they applied. The *in vivo* assays were followed by a parallel set of *ex vivo* assays using isolated rat ileum segments. In earlier studies, it has been observed that the spasmolytic action of medicinal herbs is often mediated via calcium channel inhibition [43–45] and/or K^+-channel activation [46]. To evaluate whether the antispasmodic action of cuminaldehyde was also mediated via the similar mode(s), they were tested on low and high K^+-mediated excitations, where cuminaldehyde suppressed both types of contractions while exhibiting significantly higher potency against low K^+ compared to its inhi-

Table 3. ADMET properties of Cuminaldehyde calculated *in silico* by pkCSM server-based program.

Property	Model Name	Predicted Value	Unit
A	Water solubility	−2.966	Numeric (log mol/L)
	Caco2 permeability	1.609	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	95.844	Numeric (% Absorbed)
	Skin Permeability	−1.196	Numeric (log Kp)
	P-glycoprotein substrate	No	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
D	VDss (human)	0.324	Numeric (log L/kg)
	Fraction unbound (human)	0.263	Numeric (Fu)
	BBB permeability	0.438	Numeric (log BB)
	CNS permeability	−1.485	Numeric (log PS)
M	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	No	Categorical (Yes/No)
	CYP1A2 inhibitor	Yes	Categorical (Yes/No)
	CYP2C19 inhibitor	No	Categorical (Yes/No)
	CYP2C9 inhibitor	No	Categorical (Yes/No)
	CYP2D6 inhibitor	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	Categorical (Yes/No)
E	Total Clearance	0.227	Numeric (log mL/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
T	AMES toxicity	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.839	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	No	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	1.7	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	2.194	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	No	Categorical (Yes/No)
	Skin Sensitisation	Yes	Categorical (Yes/No)
	T.Pyriformis toxicity	0.766	Numeric (log μg/L)
	Minnow toxicity	0.819	Numeric (log mM)

ADMET, absorption, distribution, metabolism, excretion, and toxicity.

bition against high K^+, thus demonstrating the involvement of predominant K^+ channel activation followed by Ca^{++} channel blockade [31]. On the other hand, cromakalim, an ATP-dependent K^+ channel opener, inhibited low K^+-mediated spasms compared to high K^+, where partial relaxation was observed, as expected [31]. However, verapamil inhibited low and high K^+-mediated contraction at comparable potencies [47,48]. To further characterize the nature of K^+-channels involved in the antispasmodic actions of cuminaldehyde, the ileum segments were preincubated with glibenclamide, which markedly reversed the suppression of low K^+, similar to cromakalim, while verapamil was found insensitive to Gb. Potassium channel openers are relatively new compounds comprised of a diverse group of molecules with a range of widespread therapeutic utility, like gastrointestinal spasms, asthma, elevated blood pressure and urinary incontinence [49–52]. These compounds cause the opening of K^+ channels and hyperpolarize the membrane, thus increase the efflux of K^+ and eventually decrease the concentration of intracellular free Ca^{++}, leading to smooth muscle relaxation [53–55]. K^+ channel opener represents

an interesting pharmacological principle with many potential clinical applications. However, most discovered drugs do not have sufficient tissue selectivity to be useful therapeutic alternatives [56].

As cuminaldehyde was also found active against high K^+-mediated contractions although at significantly lower potency, and a substance that reverses high K^+ (>30 mM)-mediated spasm is depicting CCBs [28]. Therefore, additional experiments were conducted to confirm further the Ca^{++} ion inhibitory action of cuminaldehyde, and the results were compared with verapamil [28]. The ileum tissues preincubation with cuminaldehyde in Ca^{++} free medium deflected the exogenously added Ca^{++}-CRCs to the right with suppression of the maximum peak, similar to verapamil, thus confirming the CCB-like action of cuminaldehyde.

Furthermore, to have a greater insight into cuminaldehyde and potassium channel activation binding, the molecular docking studies with the three receptors, namely 7S61, 6C3O, and 7MJP, proved to be of great importance. Cuminaldehyde has exhibited binding affinity to these receptors

comparable to the cromakalim. In prima facie, the binding energy of cuminaldehyde was lesser than cromakalim, imparting the impression that the former has a lesser affinity. However, when the residues, binding interactions, and bond distances were considered, cuminaldehyde outweighed the molecular interaction. For instance, in docking with the receptor 7S61, the cromakalim showed only one conventional hydrogen bonding with a distance of 2.67 with the residue Ser-409. At the same time, the cuminaldehyde exhibited three hydrogen bonds {1.91 (Ser721), 2.32 (Lys720), 2.48 (Ser722)} with much lesser distance. Likewise, in docking with 6C3O, cromakalim showed conventional hydrogen bonding but with a distance of 2.99. At the same time, the cumin aldehyde exhibited Wonderwall interactions with the distances 2.07 and 2.93 (Arg766), which again proved cumin aldehyde to be a better alternative. Moreover, let us compare the size of the two molecules. Cuminaldehyde is a much smaller molecule, having only one hydrogen acceptor and without a donor. At the same time, the cromakalim consists of five hydrogen bond acceptors and one donor, imparting higher chances of interaction with the residues of an active pocket of KATP. This also explains why cuminaldehyde, being a smaller molecule, exhibited significant potassium channel activation.

5. Conclusions

These findings show that cuminaldehyde possesses antidiarrheal and antispasmodic effects predominantly mediated by activation of ATP-sensitive K^+ channels followed by its additional inhibitory action on voltage-gated Ca^{++} channel. The computer docking *in silico* results also support our current findings, however, further sophisticated molecular assays are recommended, and the involvement of additional mechanism(s) must be addressed.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

MNA, NUR, and WA designed the research study. MNA, NUR, AS, and WA performed the research. NUR and AS analyzed the data. MNA, NUR, AS, and WA wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study has obtained approval from the Bio-Ethical Research Committee (BERC) at PSAU, with the reference number BERC-004-12-19.

Conflict of Interest

The authors declare no conflict of interest.

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