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Antispasmodic activity and mechanism of action of polyherbal formulation DCD-684 on rabbit jejunum

Talat Roome[1]*, Maha Qasim[1,2], Ahsana Dar Farooq[2,3], Qibtiya Ilyas[1], Sabahat Aziz[1,2] and Syed Farooq Ali[2]

[1]Section of Molecular Pathology, Department of Pathology, Dow Diagnostic Reference and Research Laboratory, Laboratory Animal Sciences, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan

[2]Medics Laboratories (Pvt.) Limited, DP 32, Sector 12C, North Karachi Industrial Area Karachi, Pakistan

[3]Hamdard Al-Majeed College of Eastern Medicine, Hamdard University, Madinat al-Hikmah, Mohammed Said Road, Karachi, Pakistan

Abstract: Digas colic drops (DCD-684) is a polyherbal formulation containing decoctions of five medicinal plants namely *Carum carvi* L., *Foeniculum vulgare* Mill*, Mentha arvensis* L., *Mentha piperita* L. and *Zingiber officinale* Roscoe. These plants have been extensively used in traditional medicine for the treatment of various gastrointestinal diseases including abdominal colic. This study was conducted to determine the spasmolytic effect of DCD-684 (100% v/v) and its individual plant components on isolated rabbit jejunum (*in vitro*) and their possible mechanism of action. The effects were evaluated on spontaneous and pre-contracted tissues using KCl (80mM) and other contractile agonists including acetylcholine (0.3µM), carbamylcholine (0.3µM), serotonin (10 µM) and histamine (100µM) in the presence and absence of DCD-684. The various concentrations of DCD-684 (0.1-3% v/v) demonstrated spasmolytic effects on both spontaneous (IC_{50}=0.75%) and KCl-induced contractions (IC_{50}=1.6%), respectively. It also inhibited the contractions induced by acetylcholine (IC_{50}=0.45%), carbamylcholine (IC_{50}=0.95%), serotonin (IC_{50}=0.95%) and histamine (IC_{50}=0.87%). The DCD-684 exhibited synergistic effect due to its five plant components suggesting that spasmolytic cascade is probably governed by muscarinic and/or nicotinic receptors, serotonergic histaminergic, as well as calcium channel blocking mechanisms. Thereby, providing the pharmacological basis of its therapeutic use in the gastrointestinal motility disorders and related inflammatory ailments.

Keywords: DCD-684, polyherbal formulation, rabbit jejunum, spasmolytic effect, cholinergic receptors, calcium channels.

INTRODUCTION

Infantile colic is a clinical condition in healthy infants suffering from paroxysmal and inconsolable crying usually during early night hours lasting for more than three hours a day or three days or a week or more than three weeks (Wessel *et al.*, 1954). It is frequently accompanied by various gestures including flushing of the face, flexing legs, clinching fists reflecting the magnitude of pain with increased peristaltic movement and excessive gas production in the distended intestines (Boero *et al.*, 1998; Sferra and Heitlinger, 1996). Infants (~10-40%) aged between two weeks to three months are predominantly susceptible to this ailment (Savino, 2007). There are several other factors related to infantile colic such as milk protein allergy or lactose intolerance (Sung, 2018) and the imbalance of intestinal hormones for example motilin, vasoactive intestinal peptide (VIP) and gastrin (Lothe *et al.*, 1987) or immature autonomic nervous system, however, its exact cause is still ambiguous (Gupta, 2007; Leung and Lemay, 2004; Savino, 2007).

Besides food and nutritional resources, whole plants and / or their different parts have also been used since antiquity as folklore medicines to combat various ailments and have incessantly played a vital role in primary health care (De Smet, 1997). According to World Health Organization, ~60% of the world's population still relies on traditional plants as herbal medicines and about 80% of the population in developing countries depends almost totally on it for their primary health care needs (Ekor, 2014; Khan and Ahmad, 2019), emphasizing its confidence and popularity among the general public. The DCD-684 polyherbal formulation manufactured by Medics Laboratories Pvt. Ltd is widely used in Pakistan for the management of infantile colic pain and other GI disturbances by the nursing mothers. Its plant components namely *Mentha piperita* L. (peppermint), *Mentha arvensis* L. (wild mint), *Carum carvi* L. (caraway), *Foeniculum vulgare* Mill (fennel) and *Zingiber officinale* Roscoe (ginger) are also popularly consumed as food and condiments. In Asian and European cuisines, mint leaves and ginger roots are used for flavoring and garnishing while caraway and fennel are popular spices used for pickling, garnishing, marinating as well as flavoring. In phytomedicine literature and traditional folklore medicine, aforementioned plants are reputed for their use

*Corresponding author: e-mail: talat.roome@duhs.edu.pk

as antispasmodic, carminative and anti-colic. Avicenna's Canon of Medicine Book 2 mentions *Z. officinale* and *F. vulgare* as digestive agents for the treatment of gastric ailments like nausea and hyperacidity (Hameed, 1998). According to the Unani Pharmacopoeia, *C. carvi*, *F. vulgare* and *Z. officinale* are part of traditional formulation *Majoon - e -Nankhwah* which is used as a digestive and carminative agent (Qazi, 2009). *F. vulgare, Z. officinale* and *Mentha. spp.* are traditionally used to treat gastrointestinal (GI) disorders like nausea, vomiting, flatulence, intestinal colic and spasms, etc (Badgujar *et al.*, 2014; Shahrajabian *et al.*, 2019; Brahmi *et al.*, 2017). Despite pharmacological evidences for each plant, there is no published report available regarding the efficacy of their combined preparation. Hence, polyherbal DCD-684 formulation (table 1) prepared as a decoction of above-mentioned plants was evaluated for *in vitro* spasmolytic activity using rabbit jejunum, a well-recognized *in vitro* model.

MATERIALS AND METHODS

Test Drugs and Chemicals

DCD-684 and individual decoctions of its five herbal components were provided by Medics Laboratories Pvt. Ltd. This DCD-684 (100%) was used to prepare various working concentrations (0.1%, 0.3%, 1% and 3% v/v) employed in subsequent experiments. The decoctions of individual herbal components (100%) were also prepared as per the quantities mentioned in table 1 and different concentrations (0.1%, 0.3%, 1%,3%, 5% and 7% v/v) were tested, respectively. Acetylcholine chloride (Ach), atropine, histamine dihydrochloride (His), carbamylcholine chloride (Cch), verapamil hydrochloride, serotonin creatinine sulfate (5-HT) and potassium chloride (KCl) were purchased from Sigma-Aldrich and Merck Chemicals, Germany. Stock solutions were prepared in deionized distilled water; however, working solutions were freshly prepared in Tyrode solution on the day of the experiment.

Animals

Albino white rabbits of either sex (1.5-2.5 kg) maintained in the animal house of Laboratory Animal Sciences (LAS), Dow University of Health Sciences, Ojha Campus, Karachi, Pakistan under standard environmental conditions of humidity (45-50%), temperature (20-24°C), with a 12 h light and dark cycle and free access to food and water were used (Seifi *et al.*, 2017). The studies were conducted according to the Institutional Animal Care and Use Committee (IACUC) and Organization for Economic Co-operation and Development (OECD) for the Testing of Chemicals (TGs) 453 guidelines. The protocol was approved by the Institutional Review Board (IRB) for Animal Research and Ethics committee of Dow University Health Sciences, Ref no: AR.IRB-013/DUHS/Approval/2018/014.

In vitro Experiments on Rabbit Jejunum

All the test substances: DCD-684 formulation, decoctions of *C. carvi, F. vulgare, M. arvensis, M. piperita and Z. officinale* as well as vehicle (sugar and glycerin, 50:1) were evaluated for *in vitro* spasmolytic activity using rabbit jejunum (*n*=5). The animals were sacrificed *via* cervical dislocation to avoid anesthesia-induced jejunum relaxation (Patel *et al.*, 2014). It was isolated from the intestinal region and kept in oxygenated Tyrode's solution for 15-30 min before the preparation of tissue segments. Tyrode's physiological salt solution comprised: NaCl (136.9mM), KCl (2.7mM), $NaHCO_3$ (11.9mM), $MgCl_2$ (1.05mM), NaH_2PO_4 (0.42mM), $CaCl_2$ (1.8mM) and glucose (5.5mM) at pH 7.4 was prepared (Seifi *et al.*, 2017; Patel *et al.*, 2014). Tissue segments (~2cm) in length were mounted vertically in tissue bath (25ml) and a resting tension (1g) was applied. The spontaneous muscular contractility was isometrically recorded (PowerLab 8/35, ADInstruments, Sydney, Australia). The tissue was maintained at 37°C with carbogen gas (Oxygen 95% and carbon dioxide 5%) and allowed to equilibrate for 30-60 minutes. After stable response, it was further monitored for 15-30 min before the addition of any test substance and changes in contractile responses were noted. The responses against spontaneous and KCl-induced contractions (80mM) were determined by cumulative addition of DCD-684 and its individual components. The effect of vehicle (sugar and glycerin) was also determined. However, for agonist induced-contractions, a single concentration of acetylcholine (0.3 µM), carbamylcholine (0.3µM), serotonin (10µM) and histamine (100 µM) were employed and inhibition in their contractions were noted. Atropine (0.1-10µM, non-selective muscarinic receptor antagonist) and verapamil (0.01-1 µM, calcium channel blocker) were also used.

STATISTICAL ANALYSIS

All data are expressed as mean ± SEM and analyzed by Statistical Package of Social Sciences (SPSS, version 17). One-way and repeated measure Analysis of Variance (ANOVA) followed by Post-Hoc LSD for multiple comparisons were performed. The *p* values less than 0.05 was considered significant. The IC_{50} and IC_{25} were calculated by linear regression analysis representing 50% and 25% inhibitory concentration, respectively.

RESULTS

The Effect of DCD-684 and its components on:

Spontaneous contractions

The jejunum spontaneous contractions (*n*=5) were noted in control or in the presence of various concentrations of DCD-684, its individual plant components, vehicle, atropine and verapamil (fig. 1 A-D and fig. 2A-C). The DCD-684 (0.1-3%) exhibited dose-dependent inhibition of 72.8±0.8% and 123.1±2.9% at 1% and 3% respectively (Supplementary table S.1A).

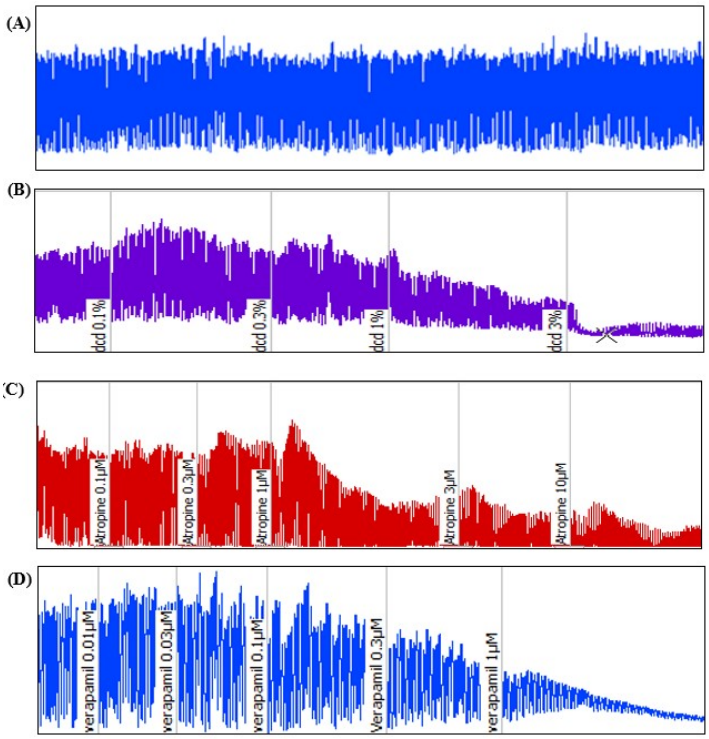

Fig. 1: Tracings showing spontaneous contractions of isolated rabbit jejunum: The upper and lower deflections indicate its contraction and relaxation, respectively. (A) Control representing 100% spontaneous contraction while the cumulative addition of (B) DCD-684 (Digas Colic Drops 0.1-3%), (C) Atropine (0.1 µM-10 µM) and (D) Verapamil (0.01 µM-1 µM) in the tissue bath 25 ml are shown accordingly. DCD-684 (100%) was used to prepare different percent concentrations (v/v).

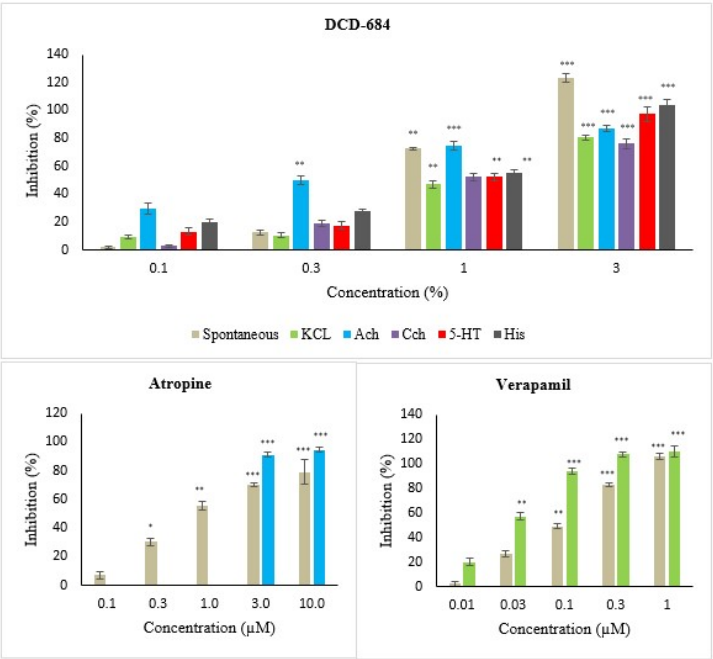

Fig. 2: Percent inhibition of Spontaneous (●) or agonist induced contractions in isolated rabbit jejunum: Potassium chloride (KCl; ● 80mM), Acetylcholine (Ach; ● 0.3 µM), Carbamylcholine (Cch; ● 0.3 µM), Serotonin (5-HT; ● 10 µM) and Histamine (His; ● 100 µM) in the presence of: DCD-684 (0.1-3%), Atropine (0.1-10 µM) and Verapamil (0.01-1 µM). The x-axis represents the concentration of the test substance while the y-axis represents the percent inhibition response. The values represent mean of percentage change ±SEM (n = 5). Asterisks indicate significant percent relaxation (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$), whereas other values were non-significant.

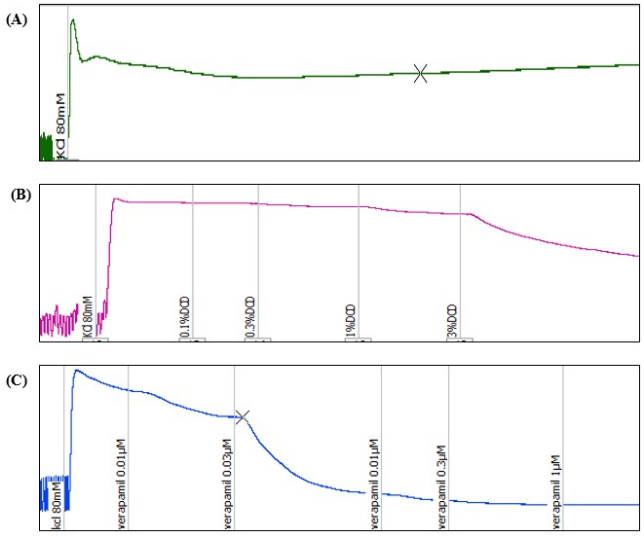

Fig. 3: Tracings showing KCl (80mM) induced contraction on isolated rabbit jejunum: (A) Control representing 100% sustained contraction while the cumulative addition of (B) (Digas Colic Drops 0.1-3%) and (C) Verapamil (0.01 µM-1 µM) in the tissue bath 25 ml are shown accordingly. DCD-684 (100%) was used to prepare different percent concentrations (v/v).

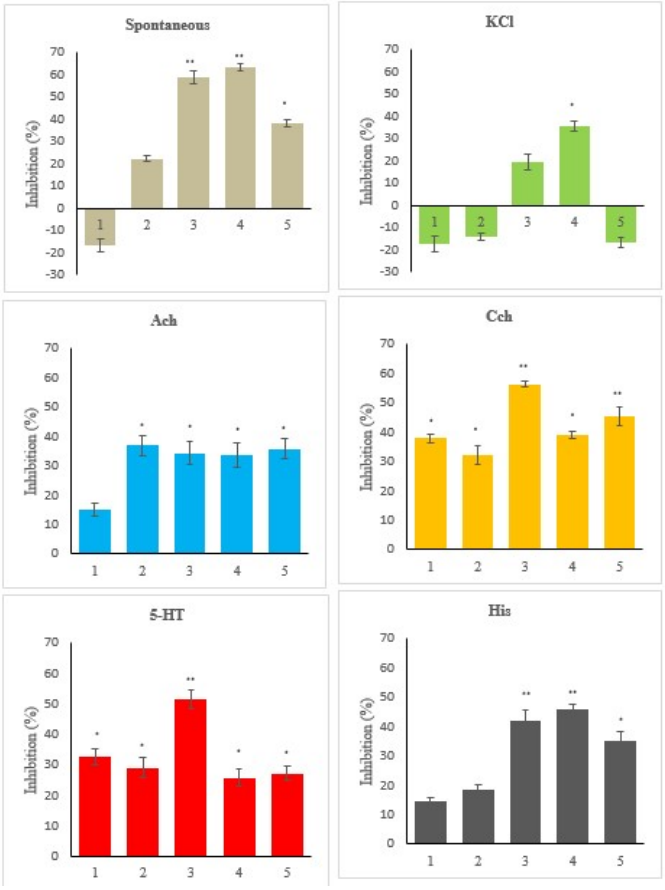

Fig. 4: Percent inhibition (%) of rabbit jejunum by DCD-684 components: (1) *C. carvi*, (2) *F. vulgare*, (3) *M. arvensis*, (4) *M. piperita* and (5) *Z. officinale* at highest concentration (7%) on Spontaneous contractions (●) or induced by Potassium chloride (KCl;●), Acetylcholine (Ach;●), Carbamylcholine (Cch;●), Serotonin (5-HT;●) and Histamine (His;●).The negative values represent contraction (%) observed on the addition of test substances with respect to control. The values represent mean of percentage change ±SEM (n = 5). Asterisks indicate significant percent relaxation (*$p < 0.05$ and **$p < 0.01$), whereas other values were non-significant.

Table 1: Composition of DCD-684 polyherbal formulation

S. No	Botanical name	Common name	Family	Part used	Quantity (mg/ml)
1.	*Carum carvi*	Caraway	*Apiaceae*	Seeds	8.00
2.	*Foeniculum vulgare*	Fennel	*Apiaceae*	Fruit	20.00
3.	*Mentha arvensis*	Wild mint	*Lamiaceae*	Stem and leaves	16.00
4.	*Mentha piperita*	Peppermint	*Lamiaceae*	Stem and leaves	16.00
5.	*Zingiber officinale*	Ginger	*Zingiberaceae*	Rhizome	6.00
	Total quantity				66

Table 2: IC_{50} and IC_{25} values of DCD-684 and its components on spontaneous and induced-contractions of rabbit jejunum

S. No	Test Substance	IC_{50} values of DCD-684 (%)					
		Spontaneous contraction	Agonists-induced contraction				
			KCl	Ach	His	5-HT	Cch
1.	DCD-684	0.75	1.6	0.45	0.87	0.95	0.95
IC_{25} values of individual components (%)							
2.	*C. carvi*	NA	NA	NA	NA	5.0	4.80
3.	*F. vulgare*	NA	NA	5.15	NA	6.25	5.60
4.	*M. arvensis*	3.80	NA	5.0	2.20	2.60	2.80
5.	*M. piperita*	0.25	5.20	5.80	2.40	6.90	3.10
6.	*Z. officinale*	3.20	NA	5.10	6.35	6.60	4.00

Table 2: Digas Colic Drops (DCD-684), Potassium chloride (KCl), Acetylcholine (Ach), Histamine (His), Serotonin (5-HT), and Carbamylcholine (Cch), Not applicable (NA). The DCD-684 alone and decoction of its each component represents 100% concentration and were used to prepare different percent dilutions (v/v). IC_{50} and IC_{25} values are the 50% and 25% inhibitory response and calculated by linear regression. The individual component with inhibitory effect less than 25% =NA.

Similarly, atropine (IC_{50}=0.92 µM) and verapamil (IC_{50}=0.12 µM) also showed dose-dependent inhibitory effect as standard drugs. Among its five individual herbal components, *M. piperita*, *Z. officinale* and *M. arvensis* demonstrated relaxant effect and their IC_{25} values are presented in table 2 while *C. carvi* and *F. vulgare* were ineffective, likewise vehicle also had no effect (Supplementary table S.1A).

KCl-induced contractions

The KCl (80 mM)-induced contractions in tissues were used to determine calcium channel blocking activity of DCD-684. The cumulative addition of DCD-684 (0.1-3%) and verapamil caused relaxation in KCl-induced contractile response in a concentration-dependent manner (fig. 2 and fig. 3 A-C). At 1% and 3%, DCD-684 showed significant inhibition of 47.1±2.78% and 80.6±1.9%. Verapamil (standard calcium channel blocker) also displayed significant relaxation response (IC_{50}=0.026µM) against high K^+. However, the vehicle had no such effect (Supplementary table S.2A and B). Among individual herbal components, only *M. piperita* showed significant inhibition of 35.8±2.3%. However, other components failed to exhibit any inhibitory response against high K^+ concentration (fig. 4B).

Acetylcholine (Ach)-induced contraction

Acetylcholine (0.3 µM) -induced spasm was reduced by the addition of DCD-684 (0.3-3%) causing 49.9±2.9%-87.1±1.9% inhibition (fig. 2A). The effect of atropine (3-10 µM) was also determined as the standard muscarinic antagonist (fig. 2B) which elicited significant inhibition of more than 90% (Supplementary table S.3A and B). The potency order of individual components against Ach-induced contractions according to their IC_{25} values (table 2) appears to be: *M. arvensis* ≥ *Z. officinale* ≥ *F. vulgare* ≥ *M. piperita*, while *C. carvi* had no such effect (fig. 4C).

Carbamylcholine (Cch)-induced contractions

Carbamylcholine (0.3 µM)-induced spasms were reduced by the addition of DCD-684 (1-3%) causing 52.2±2.3%-76.0±3.2% inhibition (Supplementary table S.6, fig. 2A). The potency of individual components considering their IC_{25} values (table 2) appears to be: *M. arvensis* > *M. piperita* > *Z. officinale* ≥ *C. carvi*> *F. vulgare* (fig. 4).

Serotonin (5-HT)-induced contraction

DCD-684 (0.1-3%) inhibited serotonin (10 µM)-induced contractions in a concentration dependent manner. DCD-684 (1%-3%) showed significant inhibition of 52.3±2.7%-97.19±5.51% (Supplementary table S.5) in the contractile response of serotonin (fig. 2A). The potency of individual components considering their IC_{25} values (table 2) appears to be: *M. arvensis* >>*C. carvi* > *F. vulgare* ≥ *Z. officinale* ≥ *M. piperita* (fig. 4).

Histamine (His)-induced contractions

Pretreatment of the intestinal tissue with DCD-684 (0.1-3%) reduced the tissue response to histamine (100 µM) in a concentration dependent manner. In the presence of

DCD-684 (1-3%), significant inhibition of 54.9±2.8-104.1±3.5% (Supplementary table S.4, fig. 2A) in the contractile response of histamine was observed. The potency of individual components of DCD-684 considering their IC_{25} values (table 2) appears to be: *M. arvensis* ≥ *M. piperita* >>*Z. officinale*. Whereas, *C. carvi* and *F. vulgare* had minimal but non-significant effect (fig. 4).

DISCUSSION

In the past few decades, there has been a massive increase in the public interest to use herbal medicines for the treatment of various ailments due to the side-effects associated with conventional medicines (Ekor, 2014; Mohamad *et al.*, 2019).Therefore, the traditional use of polyherbal medicines or phytomedicines in various countries including Pakistan, Iran, India and Kingdom of Saudia Arabia are popular and well-practiced against various diseases including gastrointestinal related ailments and diarrhea (Hajhashemi *et al.*, 2000; Mujumdar *et al.*, 2000; Ullah *et al.*, 2020; Buso *et al.*, 2020). DCD-684 has been formulated by considering the effectiveness of its plant components used popularly in traditional medicine against GI diseases including abdominal colic pains.

Moreover, these plants have been proven scientifically for their antispasmodic, carminative and digestive properties in children. For example, *Mentha. spp.* possessing peppermint oil are traditionally used against nausea, vomiting, flatulence, intestinal colic and spasms of the colon and bile duct, esophageal reflux, heartburn etc. (Brahmi *et al.*, 2017; Mullin, 2020). Interestingly, *M. piperita* is a popular single therapeutic component for many herbal antispasmodic preparations useful for treating intestinal colic, biliary disorders, spasms of the gallbladder and GI tract (McKay and Blumberg, 2006). While, *M. arvensis* is an important component in various polyherbal formulations due to its substantial carminative and antimicrobial properties (Thawkar, 2016). It is well established that menthol, an active chemical constituent residing in various *Mentha. spp.* possesses antispasmodic activity interfering with cationic influx through 5-HT_3 receptors and calcium ion channels, its anticholinergic effect has also been demonstrated in rat ileum and human colon (Heimes *et al.*, 2011; Amato *et al.*, 2014). The use of *Z. officinale* is associated with nausea induced motion sickness and also as appetite stimulant (Yassin *et al.*, 2012). Its constituents namely 6-gingerol and 6-shogaol have been reported for antispasmodic effect *via* blockade of 5-HT_3 and M_3 receptors in the isolated guinea-pig ileum (Abdel-Aziz *et al.*, 2006).The effectiveness of *C. carvi* against indigestion accompanied by mild cramps in the GI tract also facilitates in relieving flatulence (Mahboubi, 2019). Its only antipode namely (-)-Carvone has been reported as antispasmodic in guinea pig ileum by reducing contractions induced by histamine, carbachol and $BaCl_2$ (Souza *et al.*, 2013). Moreover, caraway fruit extract stimulated the proximal and distal stomach motility in the guinea pig (Krueger *et al.*, 2020). Furthermore, antagonism of irritable colon/stomach and diarrheal conditions by use of *F. vulgare* in folklore and Ayurvedic medicine system has been emphasized (Badgujar *et al.*, 2014). Its essential oils possess carminative, antispasmodic, diuretic and stomachic properties (Ibrahim *et al.*, 2020). Though, the effect of its major constituent, anethole against gut tissue contractions has not been reported whereas, its evidence-based potential against vascular smooth muscle contractility on isolated rat aorta involving voltage dependent Ca^{+2} channels has been documented (Soares *et al.*, 2007).

There are several antispasmodic drugs belonging to the class of anticholinergic and Ca^{2+} channels blockers affecting the gastrointestinal (GI) system by their action on smooth muscles and/or by modulating the activity of the enteric nervous system (ENS) embedded in the GI tract lining. The neurotransmitters acetylcholine, serotonin, histamine and various peptides including opioids are important regulators of gut motility and water absorption (Goyal and Hirano, 1996; Martínez-Pérez *et al.*, 2018). GI smooth muscles are autonomous and generate spontaneous electrical rhythmicity and contractions in the absence of neuronal or hormonal stimulation. These contractions are initiated by the activity of the interstitial cell of Cajal (ICC) which lies between the muscle layers. ICCs are electrically coupled to smooth muscle cells and initiate membrane depolarization and intrinsic pacemaker potentials referred to as slow regular waves (Sanders *et al.*, 2012; Montgomery *et al.*, 2016). The DCD-684 attenuated the spontaneous contractions of rabbit jejunum in a concentration-dependent manner which was reversible after washing the tissue with Tyrode's solution. This spasmolytic effect of DCD-684 is probably due to synergistic effects of its herbal components *via M. piperita, M. arvensis* and *Z. officinale* supporting their interaction with Ca^{2+} channels leading to membrane depolarization.

To determine the spasmolytic activity of DCD-684 by its interaction on different receptors and/or with Ca^{2+} channels, various agonists were employed in jejunum tissue. It is well-documented that KCl (80mM) induces smooth muscle contractions *via* membrane depolarization causing Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels without receptor stimulation (Cortes *et al.*, 2006; Godfraind *et al.*, 1986; Hajagos-Tóth *et al.*, 2009) and were blocked by verapamil, a reputable calcium channel blocker (Ali *et al.*, 2017). Hence, substances inhibiting such contractions may be considered as blocker of calcium influx (Godfraind *et al.*, 1986). DCD-684 also elicited relaxation of KCl-induced contractions in

jejunum. Among its individual herbal components, only *M. piperita* demonstrated significant blockade of Ca^{2+}channels supporting its earlier studies on the muscular actions and secretory processes of the GI tract as examined in various animal models. Moreover, a reduction in calcium influx has also been associated with *M. piperita* (Hills and Aaronson, 1991; McKay and Blumberg, 2006).These results indicate that DCD-684 has calcium channel blocking activity against KCl-induced contractions mainly due to the presence of menthol residing in *Mentha spp.*

It is well established that acetylcholine (Ach) increases gastrointestinal contractions by stimulating muscarinic receptors of M_3 sub-type residing in the intestinal smooth muscle *via* cellular signaling cascade (Weiser *et al.*, 1997). Briefly, the alpha subunit of the Gq/11 protein activates the effect or phospholipase C(PLC), which increases the inositol triphosphate (IP3) second messenger responsible for releasing calcium from the sarcoplasmic reticulum. This Ca^{2+} release activates voltage-gated Ca^{2+} channels indirectly facilitating the influx of Ca^{2+} from extracellular fluids (Caulfield, 1993; Eglen, 1996; Catterall *et al.*, 2005; Honda *et al.*, 1996). Likewise, carbamylcholine (Cch), a stable analog of acetylcholine also stimulates peristalsis of the GI tract by activating muscarinic cholinergic receptors, and also activates α7 subunits of cholinergic nicotinic receptors on macrophage and endothelial cells leading to cellular deactivation and inhibition of cytokine release, thereby attenuating the systemic or regional inflammatory responses (Pavlov *et al.*, 2003). Consequently, the spasmolytic action of DCD-684 on Ach and Cch-induced contractions may probably be due to its interaction with muscarinic and nicotinic receptors, respectively. The individual components of DCD-684 including *F. vulgare, M. arvensis, M. piperita* and *Z. officinale* diminished the Ach and Cch induced contractile effect. However, *C. carvi* demonstrated significant inhibitory only in Cch-induced contractions. Nevertheless, spasmolytic effect of herbal components of DCD-684 and its combined formulation validates its action on cholinergic receptors due to the presence of spasmolytic components residing in each plant.

The GIT is also richly occupied with histaminergic receptors (Ali *et al.*, 2017) and histamine plays a vital role in the regulation of gastric acid and ion secretion, gastric mucosal defense and intestinal motility (Fabisiak *et al.*, 2017). It stimulates the contractile response by increasing chloride secretion by colonic epithelium by the activation of H_1 receptors residing in the smooth muscles. Histamine may induce excitation of enteric neurons through activation of all four histamine receptors (H_1-H_4 receptors) (Coruzzi *et al.*, 2012). The relaxant effect of DCD-684 and its individual components on histamine-induced contractions mainly involves *M. arvensis, M. piperita* and *Z. officinale,* signifying its histamine receptor-mediated response on isolated rabbit jejunum. Whereas, *C. carvi* and *F. vulgare* had a minimal effect. This anti-histaminergic response of *Z. officinale,* has also been reported in previous studies (Suva, 2013) which has been associated with the presence of 6-gingerol and 6-shogaol. This spasmolytic potential of individual herbal components appears to be playing primary and cumulative role in DCD-684 induced histaminergic antagonism.

Serotonin (5-HT) is an enteric neurotransmitter mediating peristaltic reflexes controlling gastrointestinal motility, visceral sensitivity and secretion involving the 5-HT_3/5-HT_4 receptor sub-types (Crowell, 2004; Terry and Margolis, 2016). It activates both intrinsic excitatory and inhibitory enteric motor neurons by stimulating cholinergic neurons releasing acetylcholine causing contraction or excite inhibitory nitrergic neurons via nitric oxide inducing relaxation in smooth muscles. Pathophysiological changes in serotonin, directly and indirectly, affects intestinal motor and secretory function leading to abnormalities like nausea, vomiting, intestinal secretion, and peristalsis (Gershon, 1999; Sikander *et al.*, 2009). Hence, it can be suggested that DCD-684 and all of its individual herbal components have shown cumulative effect in blocking spasm possibly *via* 5-HT_3/5-HT_4 receptors with major contribution of *M. arvensis* and *C. carvi* most likely due to the presence of menthol and (-)-Carvone.

Thus, the claim made by the various traditional systems of medicine regarding the use of aforementioned herbal components in the treatment of colic pain and various GI ailments remain unchanged when used as polyherbal DCD-684 formulation. Its diverse spasmolytic action is evident by its action targeting multiple receptors and non-receptor pathways associated with gastrointestinal spasms. Among all the plant components of DCD-684, *M. arvensis, M. piperita* and *Z. officinale* demonstrated maximum efficacy towards receptor-mediated contractions, while *C. carvi* and *F. vulgare* produced a combined relaxing effect. However, its effect at the cellular and molecular level needs to be explored further supporting its spasmolytic mechanism.

CONCLUSION

The present findings led us to conclude that the penta-herbal formulation DCD-684 has a significant *in vitro* muscle relaxant effect on isolated rabbit jejunum *via* both non-receptor (calcium channel) as well as multiple receptor-mediated contractions. This study supports the efficacy and therapeutic use of DCD-684 in the management of infantile colic pain and various gastrointestinal disorders. The individual herbal components also demonstrated spasmolytic effect thereby favoring their significant synergistic contribution in this novel formulation.

Supporting Information

Table S.1A: Effect of DCD-684 and its components on spontaneous contraction of rabbit jejunum

S. No.	Test substance	Percent change in rabbit jejunum					
		Concentration (%) v/v					
		0.1	0.3	1.0	3.0	5.0	7.0
1.	DCD-684	2.1±1.0	12.6±1.6	72.8±0.8***	123.1±2.9***	---	---
2.	Vehicle	-2.7±2.3	-5.3±1.9	-11.1±2.5	-11.1±2.5	---	---
Individual components (decoction)							
1.	*C. carvi*	-5.2±1.7	5.6±2.1	-2.1±1.8	-6.3±1.5	-12.4±1.9	-16.7±2.7
2.	*F. vulgare*	-8.2±2.6	-4.7±0.9	4.3±2.8	16.2±2.9	20.8±0.9	22.2±1.1
3.	*M. arvensis*	-1.3±0.7	-4.6±2.2	6.4±1.4	16.2±3.3	35.7±2.8*	58.7±2.8**
4.	*M. piperita*	-7.6±0.8	28.0±2.8	32.6±2.0	38.1±1.7*	56.8±3.3**	63.3±1.5**
5.	*Z. officinale*	-3.8±1.9	4.7±2.2	14.7±2.6	25.5±2.5	32.2±0.8	38.0±1.5*

Table S.1B: Effect of atropine and verapamil on spontaneous contraction of rabbit jejunum

Test Substance	Concentration (µM)						
	0.01	0.03	0.1	0.3	1	3	10
Atropine	--	--	7.09±2.62	30.31±2.51*	55.45±2.81**	70.25±1.48***	79.05±8.57***
Verapamil	2.44±2.10	26.90±2.80	49.27±2.44**	83.06±1.43***	105.73±2.61***	---	---

Digas Colic Drops: DCD.

Control: Spontaneous contractions in rabbit jejunum before addition of test compound (100%).

The values presented are mean of percentage change ±SEM (n= 5) of spontaneous contractions in the presence of test compounds as compared to control (0.084-2.186 mV).

The values without sign indicate percent relaxation in rabbit jejunum, while negative sign (-) represents contraction (%) with respect to control.

The concentrations (% v/v) mentioned in the experiment are prepared by taking different volumes from 100% test substances and were maintained in 25ml of tissue bath.

Asterisks indicate significant percent relaxation (*p<0.05, **p<0.01 and *** p<0.005), whereas other values were non-significant.

Table S.2A: Effect of DCD-684 and its components on KCl-induced contraction of rabbit jejunum

S. No	Test substance	Percent change in rabbit jejunum					
		Concentration (%) v/v					
		0.1	0.3	1.0	3.0	5.0	7.0
1.	DCD-684	9.6±1.2	10.6±1.4	47.1±2.78**	80.6±1.9***	---	---
2.	Vehicle	28.7±1.9	-13.2±1.7	-7.6±2.4	-3.4±2.2	---	---
Individual components (decoction)							
1.	*C. carvi*	-1.8±1.9	-2.3±2.5	-2.8±2.9	-3.6±3.1	-8.7±2.3	-17.6±3.5
2.	*F. vulgare*	3.8±2.2	2.1±1.3	2.5±1.5	-0.7±0.1	-3.8±1.1	-14.3±1.6
3.	*M. arvensis*	3.3±2.2	8.1±1.4	13.4±2.6	16.6±2.7	18.1±3.6	19.2±3.5
4.	*M. piperita*	-2.0±1.1	-8.6±0.7	-11.3±0.7	-15.9±2.1	24.1±2.7*	35.8±2.3*
5.	*Z. officinale*	1.1±1.6	2.2±0.3	-0.2±0.9	-0.4±2.3	-13.0±1.7	-16.8±2.2

Table S.2B: Effect of verapamil on KCl-induced contraction of rabbit jejunum

	Concentration (µM)				
	0.01	0.03	0.1	0.3	1
Mean relaxation % ±SEM	20.2±2.8	57.2±3.3**	93.8±2.7***	107.5±2.4***	110.0±4.9***

Digas Colic Drops: DCD.

Control: The sustained contraction evoked by KCl in rabbit jejunum before addition of test compound (100%).

The values presented are mean of percentage change ±SEM (n= 5) of KCl induced contraction in the presence of test compounds as compared to control (0.216-3.695 mV).

The values without sign indicate percent relaxation in rabbit jejunum, while negative sign (-) represents contraction (%) with respect to control.

The concentrations (% v/v) mentioned in the experiment are prepared by taking different volumes from 100% test substances and were maintained in 25ml of tissue bath.

Asterisks indicate significant percent relaxation (*p<0.05, **p<0.01 and *** p<0.005), whereas other values were non-significant.

Table S.3A: Effect of acetylcholine in the presence of DCD-684 and its herbal components

S. No.	Test substance	Percent change in rabbit jejunum					
		Concentration (%) v/v					
		0.1	0.3	1.0	3.0	5.0	7.0
1.	DCD-684	29.6±4.2	49.9±2.9**	74.68±2.7***	87.1±1.9***	---	---
Individual components (decoction)							
1.	*C. carvi*	1.85±1.31	-10.1±3.3	-4.8±2.9	3.6±2.0	9.4±0.8	15.2±2.3
2.	*F. vulgare*	5.84±1.07	8.3±0.9	8.6±0.7	16.3±1.7	24.2±2.4	37.0±3.4*
3.	*M. arvensis*	7.67±2.50	-3.4±0.8	0.7±0.6	10.1±3.1	26.2±3.5	34.4±4.1*
4.	*M. piperita*	7.67±2.50	-3.4±0.8	0.7±0.3	6.4±3.8	19.6±1.9	33.6±4.1*
5.	*Z. officinale*	2.20±1.92	4.5±1.7	6.7±1.9	15.7±1.0	21.9±2.	35.7±3.4*

Table S.3B: Effect of acetylcholine in the presence of atropine

Test substance	Percentage relaxation of atropine on rabbit jejunum	
	Concentration (μM)	
	3	10
Atropine	91.16±2.00***	94.52±1.67***

Table S.4: Effect of histamine in the presence of DCD-684and its herbal components

S. No.	Test substance	Percent change in rabbit jejunum					
		Concentration (%) v/v					
		0.1	0.3	1.0	3.0	5.0	7.0
1.	DCD-684	19.9±2.6	27.7±1.8	54.9±2.8**	104.1±3.5***	---	---
Individual components (decoction)							
1.	*C. carvi*	1.9±0.3	7.4±1.7	7.9±1.7	10.8±0.7	13.0±2.4	14.7±1.3
2.	*F. vulgare*	2.7±2.4	0.2±1.5	4.4±1.8	10.7±1.3	14.2±1.3	18.5±1.9
3.	*M. arvensis*	3.3±2.2	10.2±1.6	20.2±3.4	28.6±2.8	36.2±2.5*	42.1±3.6**
4.	*M. piperita*	3.9±2.0	16.1±3.5	20.5±2.4	25.8±3.3	36.5±1.9*	46.1±1.6**
5.	*Z. officinale*	-16.6±2.9	-16.5±3.0	-18.4±3.2	-22.1±2.2	4.8±1.4	35.1±3.4*

Table S.5: Effect of serotonin in the presence of DCD-684and its herbal components

S. No.	Test substance	Percent change in rabbit jejunum					
		Concentration (%) v/v					
		0.1	0.3	1.0	3.0	5.0	7.0
1.	DCD-684	13.3±3.0	17.5±2.5	52.3±2.7**	97.19±5.51***	---	---
Individual components (decoction)							
1.	*C. carvi*	4.2±2.5	9.6±2.2	12.9±2.6	16.82±2.74	25.5±2.5	32.6±2.7*
2.	*F. vulgare*	-11.5±7.0	-4.5±1.4	-1.6±2.7	4.34±1.24	18.3±1.8	29.1±3.4*
3.	*M. arvensis*	7.1±3.0	10.4±2.4	15.1±2.5	30.8±3.1	45.1±3.1*	51.6±3.0**
4.	*M. piperita*	-9.8±1.6	-8.4±1.9	-7.4±1.6	6.8±2.3	16.1±2.8	25.8±2.9*
5.	*Z. officinale*	-13.9±1.0	-20.1±4.6	-20.9±1.8	-24.1±1.0	16.2±3.5	27.2±2.4*

Digas Colic Drops: DCD.
Control: The sustained contraction evoked by Histamine in rabbit jejunum before addition of test compound (100%).
The values presented are mean of percentage change ±SEM (n= 5) of Histamine induced contraction in the presence of test compounds as compared to control (0.264-2.158 mV).
The values without sign indicate percent relaxation in rabbit jejunum, while negative sign (-) represents contraction (%) with respect to control.
The concentrations (% v/v) mentioned in the experiment are prepared by taking different volumes from 100% test substances and were maintained in 25ml of tissue bath.
Asterisks indicate significant percent relaxation (*p<0.05, **p<0.01 and *** p<0.005), whereas other values were non-significant.

Table S.6: Effect of carbamylcholine in the presence of DCD-684and its herbal components

S. No	Test substance	Percent change in rabbit jejunum					
		Concentration (%) v/v					
		0.1	0.3	1.0	3.0	5.0	7.0
1.	DCD-684	2.81±0.7	19.2±2.2	52.2±2.3**	76.0±3.2***	---	---
Individual components (decoction)							
1.	*C. carvi*	-7.1±3.01	-14.5±2.1	-22.7±5.7	-28.4±6.2	29.3±3.2	38.0±1.5*
2.	*F. vulgare*	-1.4±2.5	3.7±2.5	5.1±2.8	13.4±2.9	20.3±2.8	32.2±3.3*
3.	*M. arvensis*	21.3±3.5	18.8±3.3	15.9±3.2	27.6±1.2	34.5±0.3*	56.4±0.9**
4.	*M. piperita*	-0.4±2.0	8.7±1.1	21.1±4.3	25.7±2.8	34.3±2.7*	39.1±1.3*
5.	*Z. officinale*	2.8±1.6	10.1±2.5	12.9±3.3	18.8±2.5	31.1±2.6*	45.2±3.2**

Digas Colic Drops: DCD.
Control: The sustained contraction evoked by Carbamylcholine in rabbit jejunum before addition of test compound (100%).
The values presented are mean of percentage change ±SEM (n= 5) of Carbamylcholine-induced contraction in the presence of test compounds as compared to control (0.230-1.466 mV).
The values without sign indicate percent relaxation in rabbit jejunum, while negative sign (-) represents contraction (%) with respect to control.
The concentrations (% v/v) mentioned in the experiment are prepared by taking different volumes from 100% test substances and were maintained in 25ml of tissue bath.
Asterisks indicate significant percent relaxation (*p< 0.05, **p< 0.01 and *** p<0.005), whereas other values were non-significant.

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REFERENCES

Abdel-Aziz H, Windeck T, Ploch M and Verspohl EJ (2006). Mode of action of gingerols and shogaols on 5-HT3 receptors: Binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. *Eur. J. Pharmacol.,* **530**(1-2): 136-143.

Ali N, Jamil A, Shah SWA, Shah I and Ahmed G (2017). Spasmogenic and spasmolytic activity of rind of Punica granatum Linn. *BMC Complement Altern. Med.,* **17**(1): 97.

Amato A, Liotta R and Mule F (2014). Effects of menthol on circular smooth muscle of human colon: Analysis of the mechanism of action. *Eur. J. Pharmacol.,* **740**: 295-301.

Badgujar SB, Patel VV and Bandivdekar AH (2014). *Foeniculum vulgare* Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *BioMed. Res. Int.,* pp.1-32.

Boero DL, Bianchi C, Volpe C, Marcello A and Lenti C (1998). Newborns crying in different contexts: Discrete or graded signals? *Percept. Mot. Skills.,* **86**: 1123-1140.

Brahmi F, Khodir M, Mohamed C and Pierre D (2017). Chemical composition and biological activities of Mentha species. Aromatic and medicinal plants-back to nature. Cairo, Egypt, pp.47-78.

Buso P, Manfredini S, Reza Ahmadi-Ashtiani H, Sciabica S, Buzzi R, Vertuani S and Baldisserotto A (2020). Iranian medicinal plants: from ethnomedicine to actual studies. *Medicina*, **56**(3): 97.

Catterall WA, Perez-Reyes E, Snutch TP and Striessnig J (2005). International union of pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol. Rev.,* **57**(4): 411-425.

Caulfield MP (1993). Muscarinic receptors-characterization, coupling and function. *Pharmacol. Ther.,* **58**(3): 319-379.

Cortes AR, Delgadillo AJ, Hurtado M, Domínguez-Ramírez AM, Medina JR and Aoki K (2006). The antispasmodic activity of *Buddleja scordioides* and *Buddleja perfoliata* on isolated intestinal preparations. *Biol. Pharm. Bull.,* **29**(6): 1186-1190.

Coruzzi G, Adami M and Pozzoli C (2012). Role of histamine H4 receptors in the gastrointestinal tract. *Front. Biosci. (Schol Ed),* **4**(1): 226-39.

Crowell MD (2004). Role of serotonin in the pathophysiology of the irritable bowel syndrome. *Br. J. Pharmacol.,* **141**(8): 1285-1293.

De Smet PA (1997). The role of plant-derived drugs and herbal medicines in healthcare. *Drugs,* **54**(6): 801-840.

Eglen RM (1996). Muscarinic receptor subtypes and smooth muscle function. *Pharmacol Rev.,* **48**(4): 531-565.

Ekor M (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol.,* **4**: 177.

Fabisiak A, Włodarczyk J, Fabisiak N, Storr M and Fichna J (2017). Targeting histamine receptors in irritable bowel syndrome: A critical appraisal. *J. Neurogastroenterol. Motil.,* **23**(3): 341.

Gershon M (1999). Roles played by 5-hydroxytryptamine in the physiology of the bowel. *Aliment. Pharmacol. Ther.,* **13**(S2): 15-30.

Godfraind T, MILLER R and Wibo M (1986). Calcium antagonism and calcium entry blockade. *Pharmacol. Rev.,* **38**(4): 321-416.

Goyal RK and Hirano I (1996). The enteric nervous system. *N. Engl. J. Med.,* **334**(17): 1106-1115.

Gupta SK (2007). Update on infantile colic and management options. *Curr. Opin. Investig. Drugs.,* **8**: 921-926.

Hajagos-Tóth J, Falkay G and Gaspar R (2009). Modification of the effect of nifedipine in the pregnant rat myometrium: The influence of progesterone and terbutaline. *Life Sci.,* **85**(15-16): 568-572.

Hajhashemi V, Sadraei H, Ghannadi AR and Mohseni M (2000). Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. *J. Ethnopharmacol.,* **71**(1-2): 187-192.

Hameed HA (1998). *Al-Qanun Fi-l-Tibb,* New Delhi Department of Islamic Studies, Jamia Hamdard (Hamdard University).

Heimes K, Hauk F and Verspohl EJ (2011). Mode of action of peppermint oil and (-)-menthol with respect to 5-HT3 receptor subtypes: binding studies, cation uptake by receptor channels and contraction of isolated rat ileum. *Phytother Res.,* **25**(5): 702-708.

Hills JM and Aaronson PI (1991). The mechanism of action of peppermint oil on gastrointestinal smooth muscle: an analysis using patch clamp electrophysiology and isolated tissue pharmacology in rabbit and guinea pig. *Gastroenterology,* **101**(1): 55-65.

Honda K, Takano Y and Kamiya HO (1996). Involvement of protein kinase C in muscarinic agonist-induced contractions of guinea pig ileal longitudinal muscle. *J. Gen. Physiol.,* **27**(6): 957-961.

Ibrahim ME, Abd Rabbu HS, Motawe HM and Hussein MS (2020). Improved growth, yield of seeds and oil production of fennel (*Foeniculum vulgare* var. vulgare) plants. *J. Mater. Environ. Sci.,* **11**(7): 1112-1120

Khan MSA and Ahmad I (2019). Herbal medicine: current trends and future prospects. New look to phytomedicine: Advancements in herbal products as novel drug leads. *Elsevier,* pp.3-13.

Krueger D, Schauffele S, Zeller F, Demir IE, Theisen J, Michel K and Schemann M (2020). Peppermint and caraway oils have muscle inhibitory and pro-secretory activity in the human intestine *in vitro. J Neurogastroenterol. Motil,* **32**(2): e13748.

Leung AK and Lemay JF (2004). Infantile colic: A review. *J R Soc Promot Health,* **124**(4): 162-166.

Lothe L, Ivarsson S and Lindberg T (1987). Motilin, vasoactive intestinal peptide and gastrin in infantile colic. *Acta Paediatr.,* **76**(2): 316-320.

Mahboubi M (2019). Caraway as important medicinal plants in management of diseases. *Nat. Prod. Bioprospect.,* **9**(1): 1-11.

Martínez-Perez EF, Juarez ZN, Hernández LR and Bach H (2018). Natural antispasmodics: source, stereo-chemical configuration and biological activity. *BioMed. Res. Int.,* pp.1-32

McKay DL and Blumberg JB (2006). A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytother. Res.,* **20**(8): 619-633.

Mohamad TAST, Islahudin F, Jasamai M and Jamal JA (2019). Preference, perception and predictors of herbal medicine use among malay women in Malaysia. *Patient Prefer. Adherence,* **13**: 1829.

Montgomery LE, Tansey EA, Johnson CD, Roe SM and Quinn JG (2016). Autonomic modification of intestinal smooth muscle contractility. *Adv. Physiol. Educ.,* **40**(1): 104-109.

Mujumdar A, Upadhye A and Misar A (2000). Studies on antidiarrhoeal activity of Jatropha curcus root extract in albino mice. *J. Ethnopharmacol.,* **70**(2): 183-187.

Mullin GE (2020). Inflammatory Bowel Disease (Crohn's Disease and Ulcerative Colitis). Textbook of Natural Medicine. Elsevier.1473-1487.

P Patel M, Kanzariya N, Soni H and Patel G (2014). Evaluation of acute toxicity and *in-vitro* anti-spasmodic activity of Caspa drops, an ayurvedic formulation. *J. Pharm. Innov.,* **3**(6, Part B): 70.

Pavlov VA, Wang H, Czura CJ, Friedman SG and Tracey KJ (2003). The cholinergic anti-inflammatory pathway: A missing link in neuroimmunomodulation. *J. Mol. Med.,* **9**(5): 125-134.

Qazi GN (2009). The Unani Pharmacopoeia of India. Central Council for Research in Unani Medicine, New Dehli, India.

Sanders KM, Koh SD, Ro S and Ward SM (2012). Regulation of gastrointestinal motility insights from smooth muscle biology. *Nat. Rev. Gastroenterol Hepatol.,* **9**(11): 633.

Savino F (2007). Focus on infantile colic. *Acta Paediatr.,* **96**(9): 1259-1264.

Seifi A, Changizi S, Khori V, Hossini F, Davarian A, Jand Y, Enayati A, Mazandarani M and Nanvabashi F (2017). Hydro-alcoholic extract of Matricaria recutita exhibited dual anti-spasmodic effect via modulation of Ca^{2+} channels, NO and PKA2-kinase pathway in rabbit jejunum. *Avicenna J. Phytomed.,* **7**(4): 334.

Sferra TJ and Heitlinger LA (1996). Gastrointestinal gas formation and infantile colic. *Pediatr. Clin North Am.,* **43**(2): 489-510.

Shahrajabian MH, Sun W and Cheng Q (2019). Clinical aspects and health benefits of ginger (Zingiber officinale) in both traditional Chinese medicine and modern industry. *Acta Agric. Scand. B Soil Plant. Sci.,* **69**(6): 546-556.

Sikander A, Rana SV and Prasad KK (2009). Role of serotonin in gastrointestinal motility and irritable bowel syndrome. *Clin. Chim. Acta.,* **403**(1-2): 47-55.

Soares PMG, Lima RF, de Freitas Pires A, Souza EP, Assreuy AMS and Criddle DN (2007). Effects of anethole and structural analogues on the contractility of rat isolated aorta: Involvement of voltage-dependent Ca^{2+}-channels. *Life Sci.,* **81**(13): 1085-1093.

Souza FVM, da Rocha MB, de Souza DP and Marçal RM (2013). (-)-Carvone: Antispasmodic effect and mode of action. *Fitoterapia*, **85**: 20-24.

Sung V (2018). Infantile colic. *Aust. Prescr.,* **41**(4): 105.

Suva MA (2013). Spasmolytic effect of ginger (*Zingiber officinale* Roscoe) on chicken intestinal preparation. *Inventi Rapid: Ethnopharmacology,* **4**: 888.

Terry N and Margolis KG (2016). Serotonergic mechanisms regulating the GI tract: Experimental evidence and therapeutic relevance. *Handb. Exp. Pharmacol. Springer,* pp.319-342.

Thawkar BS (2016). Phytochemical and pharmacological review of *Mentha arvensis. Int. J. Green Pharm.,* **10**(2): 71-75.

Ullah R, Alqahtani AS, Noman OM, Alqahtani AM, Ibenmoussa S and Bourhia M (2020). A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. *Saudi J. Biol. Sci.,* **27**(10): 2706.

Weiser M, Mutschler E and Lambrecht G (1997). Characterization of postjunctional muscarinic receptors mediating contraction in rat anococcygeus muscle. *Naunyn Schmiedebergs Arch. Pharmacol.,* **356**(5): 671-677.

Wessel MA, Cobb JC, Jackson EB, Harris GS & Detwiler AC (1954). Paroxysmal fussing in infancy, sometimes called "colic". *Pediatrics,* **14**(5): 421-435.

Yassin NA, El-Rokh E-SM, El-Shenawy SM and Ibrahim BM (2012). The study of the antispasmodic effect of ginger (*Zingiber officinale*) *in vitro. Der Pharm. Lett.,* **4**(1): 263-274.