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Antimotility, Anti-Diarrheal, and Antibacterial Activities of Hydroalcoholic Extract of *Cordia Dichotoma* Leaves in Wistar Rat

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Abstract

Antidiarrheal activities of the hydro-alcoholic extract of *Cordia dichotoma* leaves (CD). The CD was screened on the dose-response curve (DRC) of acetylcholine (Ach) over isolated ileum was evaluated in the model (n=6), including Group I received 0.1% Carboxy-methyl cellulose@1ml / 100g b.wt., Group II, Loperamide @2.5 mg / Kg b.wt., Group III, IV, and V, had CD @125, 250, and 500 mg / Kg respectively. The CD was also screened for its antibacterial effects over *Escherichia coli* and *Staphylococcus aureus.* CD shifted the DRC of Ach towards the right, showed a dose-dependent decline in diarrhea, and had antibacterial activities over *E. coli.*

Key words: *Cordia dichotoma*, anti-diarrheal, Dose-response curve

Diarrhea is a serious concern to the livestock sector. The morbidity including the span consumed on care and treatment as well as periods of unproductive phase of growth and performance and sometimes mortality (Bazeley, 2003) has serious economic consequences for the farmers. Acute diarrhea is usually caused by bacteria (e.g., *Salmonella, Shigella,* and *Escherichia coli*) and viruses (e.g., Rotavirus). On the other hand, chronic diarrhea is usually related to a functional disorder such as irritable bowel syndrome or an intestinal disease. In addition, diarrhea may be persistent or chronic owing to parasitic infections due to *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium* (Thapar and Sanderson, 2004). It is estimated by the WHO that around 80% of the population uses traditional medicines and about 85% of traditional medicine includes plants. India is known to have safely and continuously utilized many herbal drugs since ages and is time tested traditional system of medicine. Scientific validation of traditional medicines has paved the way for the development of modern medicine. The quest for safe and more effective agents from plant origin has continued to be an important area of active research. Common masses residing in developing countries do trust the time-tested traditional medicine to treat diverse ailments including diarrhea (Park, 2015).

Cordia dichotoma is one of the traditional medically important plants available all over India. Traditionally, leaves of the plant have been used as antimicrobial (Khond *et al*., 2009), anthelmintic, astringent, and demulcent along with various other ailments. Further, other parts like seeds, fruit, bark, and roots including leaves are also known to have medicinal uses *viz.* anti-dysentery, anti-dyspepsia, anthelmintic, anti-larvicidal, hepato-protective, immuno-

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modulator and anti-inflammatory activities (Patel *et al*., 2011). Locally in Jammu, India, these plant leaves are being used traditionally by local people as fodder to tackle diarrhea among cattle and livestock, especially in summers and early winters. Owing to the paucity of literature regarding the antidiarrheal activities of *Cordia dichotoma*, the study was designed to investigate its antidiarrheal activity in a laboratory model.

Materials and Methods

Procurement of herb, animals, and chemicals

The selected plant material was collected from the District Reasi of Jammu Province, India, authenticated as *Cordia dichotoma* G. Forst (Lasura) by the Herbarium, Botany Department, University of Jammu, deposited as voucher specimens vide accession number 15763. The leaves were washed, shade dried, pulverized, and subjected to 50:50 hydroalcoholic extraction (CD) in the ratio of 1:10, in brief, the mixture was macerated with occasional daily shaking, filtered, centrifuged, and dried with the help of a rotary evaporator.

Albino Wistar rats weighing 150 to 160 grams were procured locally from IIIM Jammu. The rats were placed in a standard milieu, at a controlled temperature (25±2 °C), an equal span of light and dark phases, and nourished with standard pellet diet and water *ad lib.* The animals were allowed to acclimatize for two weeks before the commencement of the study. All experimental protocols were approved by the Institutional Animal Ethics Committee constituted as per the Committee for the Purpose of Control and Supervision on experiments on Animals (CPCSEA registration no. 862/GO/Re/S/04/CPCSEA).

Acetylcholine chloride (HiMedia Laboratories Pvt. Ltd., Mumbai, India) stock solution was prepared afresh. Only freshly prepared Tyrode solution (pH 7.3 to 7.4) was used for smooth muscle preparation. Carboxymethyl cellulose (CMC) and all the chemicals used for the Tyrode solution were of analytical grade and procured from HiMedia Laboratories Pvt. Ltd, Mumbai, India. Loperamide (Sigma-Aldrich Chemie GMbH, Germany), Ethanol (Merck KGaA, Germany), Demineralized water (Qualikems Fine Chem Pvt. Ltd., Vadodara, India), and Castor oil (SD Fine-chem. Limited, Mumbai, India) were procured, respectively.

For antibacterial properties of CD, on bacteria *viz. Escherichia coli (ATEC)* and *Staphylococcus aureus (MRSA)* obtained from Division of Microbiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R. S. Pura, Jammu, J&K, India were used and experiment was conducted in the Mueller Hinton Agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) following all microbiological safety precautions.

Smooth muscle preparation and assembly

Wistar rats weighing 150-160 grams were euthanized using urethane after a fasting period of 18 hours. The ileum was sectioned in two-centimeter pieces and transferred to a dish containing a Tyrode solution (NaCl, 8gm; KCl, 0.2gm; $MgCl_2$, 0.1 gm; CaCl2, 0.2 gm; $NaHCO_3$, 1.0 g; NaH_2PO_4, 0.05 gram; $C_6H_{12}O_6$, 1.0 g per liter). The segments were freed of any mesenteric attachments. The contents of ileum were washed gently with Tyrode solution using a syringe. Both ends of the ileum were tied to metal hooks taking the precaution not to occlude the lumen and that its patency was maintained. Tied segments of the ileum were suspended in a thermo-regulated 10 ml organ bath, containing Tyrode solution.

One end of the ileum was attached to a tissue holder at the base of the organ bath and the other end to the isometric recording device (transducer). The temperature was maintained at 34±1°C and allowed to equilibrate for 30 minutes. The tissue was constantly bubbled with an air mixture of 95% O_2 and 5% CO_2. The preparation was washed every 10 minutes or after allowing a sufficient contact period with Tyrode solution. Different chemicals (acetylcholine, potassium chloride) were used for inducing contractions and the effect of the *Cordia dichotoma* leaf extract was observed on the contraction of rat ileum using a data acquisition system (PowerLab, ADInstruments, Australia) and a comparison was made between the contraction caused by acetylcholine and different doses of

the hydroalcoholic extract of *Cordia dichotomy leaves* (CD)on the isolated rat ileum.

Effect of CD on dose-response curve of acetylcholine

The stock solutions of acetylcholine were prepared in distilled water, serially diluted with physiologic saline solution afresh, just before the commencement of the experiment. The final concentrations of CD were conferred as 0.01µg,0.1µg, and 1µg, per ml when mixed in a 10 ml organ bath solution, intended for organ exposure. The time cycle for these preparations was: baseline recording (0-30 s), the response of acetylcholine, *i.e.* contact time (30Sec.), and wash (60Sec.). The response of acetylcholine was taken with different concentrations of the Ach and EC_{50} value was calculated.

Once response was observed and confirmed with the increasing doses of acetylcholine, the tissue (ileum) was washed with Tyrode solution, and left to resume its normal contraction. After a stabilized regular contraction, the tissue was incubated with different concentrations of the CD (0.01 µg/ml, 0.1 µg/ml, 1 µg/ml) for half an hour and again concentration-response curve was established in the presence of different concentrations of the ACh. Contractions were again recorded in the same manner as mentioned earlier. Thus, from these graphs, the height of contractions was measured and converted into percentage responses with respect to the maximum contraction of the log molar concentrations of the dose of acetylcholine (Ach) in absence and presence of different concentrations of the CD (1 µg/ml, 0.1 µg/ml, 0.01 µg/ml). Following this mean, the standard error of the mean (SEM) of the percentage response was obtained (Yuan *et al*., 2011). Finally, the effect of CD in terms of its effect on contraction or tension on isolated ileum was predicted, and Emax was obtained.

Data are expressed as a percentage of the maximum contraction obtained from the corresponding control curve. Each point represents the mean ± SEM of six experiments for the dose of the CD extract. The acetylcholine dose-response curves, with and without the different doses of CD, were plotted using GraphPad Prism software, version 4.1. Schiffe post-hoc test was used for analysis of *in vitro* data. The Effective Dose 50 (ED_{50}) was calculated by the probit analysis (Prism4.1). The data were considered statistically significant when P-value was less than 0.05. The mean concentration-response curve to contraction rate was analyzed using non-linear regression of GraphPad Prism software (GraphPad Software Inc., San Diego, USA).

In vivo studies

The Wistar albino rats were arbitrarily allocated into five groups of six rats each. The animals were starved for18 hours prior to the experiments. Group I acted as negative control and received 0.1% CMC @ 1 ml/100g b.wt. Group II acted as positive control, treated withLoperamide@2.5mg/Kg b.wt. Group III, IV and V acted as diarrhea test groups administered with CD @ 125 mg/Kg, 250 mg/Kg, and 500 mg/Kg b.wt. respectively. The time of respective groupwise drug administration was recorded. Sixty minutes following drug treatment, each animal was administered castor oil orally at 1 ml/100 g body weight as reported (Tunaru *et al*., 2012). The rats were then placed in their respective metabolic cages. The latent period (the time between castor oil administration and appearance of first diarrheic drop) was recorded. Observation of defecation was then continued up to 5 hours on filter paper beneath the individual perforated rat cages after the appearance of the first fecal droppings for each group. This paper was replaced intermittently every hour after noting its weight. The percentage inhibition of defecation was evaluated as below:

$$\text{Inhibitionofdefecation (\%)} = \frac{(Mc - Md)}{Mc} X100$$

Where Mc=Mean number of defecations caused by castor oil,

Md=Mean number of defecations after administration of the extract

The difference between me ans of the same parameter were determined by two-way ANOVA (Snedecor and Cochran, 1994) and the result was considered statistically significantat95% confidence interval (P<0.05).

Antibacterial activity of CD

Antibiotic susceptibility was done using the Kirby-Bauer test (Hombach *et al*., 2013). The presence or absence of zones of inhibition (ZOI) around the disc, identifies the bacterial sensitivity to the drug. The diameter of the ZOI was measured with zone reader and the results of such an experiment constituted an antibiogram. Triplicate sets of disks were used for antibacterial screening. The respective test sets of discs were prepared to confer CD concentrations of 250, 500, and 1000 µg respectively on each 6 mm disc. The other sets of discs were of antibiotics *viz.* ampicillin, chloramphenicol, enrofloxacin and gentamicin. These antibiotics were considered as positive control in order to assure their action as standard against the test organisms (*Escherichia coli* and *Staphylococcus aureus*) and for comparison of their response with that produced by test samples of CD. The final set was represented by blank discs and used as a negative control.

A uniform suspension of sterilized media inoculated with respective test organism in log phase (18hrs culture in broth) was prepared at 45°C under an aseptic ambiance. The Mueller Hinton agar plus bacterial suspensions were immediately transferred to the sterile Petri dishes (100mm) to have a uniform depth of approximately 4mm and left at room temperature to solidify for 10 minutes. All the three sets of disks (test sample, standard and blank) were then put to place using a pair of sterile forceps on the seeded media plates and kept at the same position for 2 hrs at room temperature to facilitate maximum diffusion followed by incubation at 37 °C for 24 hrs to facilitate bacterial growth. Later, following incubation, the zone of inhibition around various disks was measured using a zone reader, while holding the plate up to the light. Results were considered as the antibacterial effect if ZOI of the bacterial growth was more than 10 mm.

Results and Discussion

Effect of acetylcholine on the isolated rat ileum

The dose response curve (DRC) of different doses of acetylcholine (Ach) and same after incubation with various concentrations of CD was studied on three animals from which two sections each of isolated ileum were used. The individual dose of CD was run on isolated ileum in triplicate which was randomized later to rule out any discrepancy. Therefore, each dose of CD was screened six times in total. The representative dose-response curve as acquired by data acquisition has been depicted in Fig 1A and the bar diagram of the same in Fig 1B.

Acetylcholine produced a dose-dependent increase in contraction of isolated rat ileum preparation. These results suggested that acetylcholine induces contractility of the intestinal strip. The concentration-response curve of acetylcholine in rat ileum (Fig. 2) shifted leftward with a higher E_{max} value (Table I).

Different doses of CD (1 µg/ml, 0.1 µg/ml, and 0.01µg/ml) shifted the DRC of Ach towards right with a change in the E_{max} value (Table I). The antagonism in terms o f do se dependent response produced by different doses of CD has been shown in Fig.1B. In addition, the values

Table I: Effect of acetylcholine and various doses of CD on the dose-response curve of rat ileum.

	Contraction (percentage basis)							
Ach [LogM]	**Control (% contraction)**		**1µg (% contraction)**		**0.1µg (% contraction)**		**0.01µg (% contraction)**	
X	**Mean**	**N**	**Mean**	**N**	**Mean**	**N**	**Mean**	**N**
-7	13.701±4.062	18	-0.127±1.119	6	1.283 ± 1.168	6	-1.192 ±1.048	6
-6	43.957±8.869	18	5.671±2.904	6	14.185 ± 5.85	6	3.88±2.405	6
-5	70.287±11.917	18	27.619±9.996	6	37.137±17.489	6	28.451±9.176	6
-4	86.457±13.739	18	67.019± 15.03	6	79.127±23.189	6	67.495±14.139	6

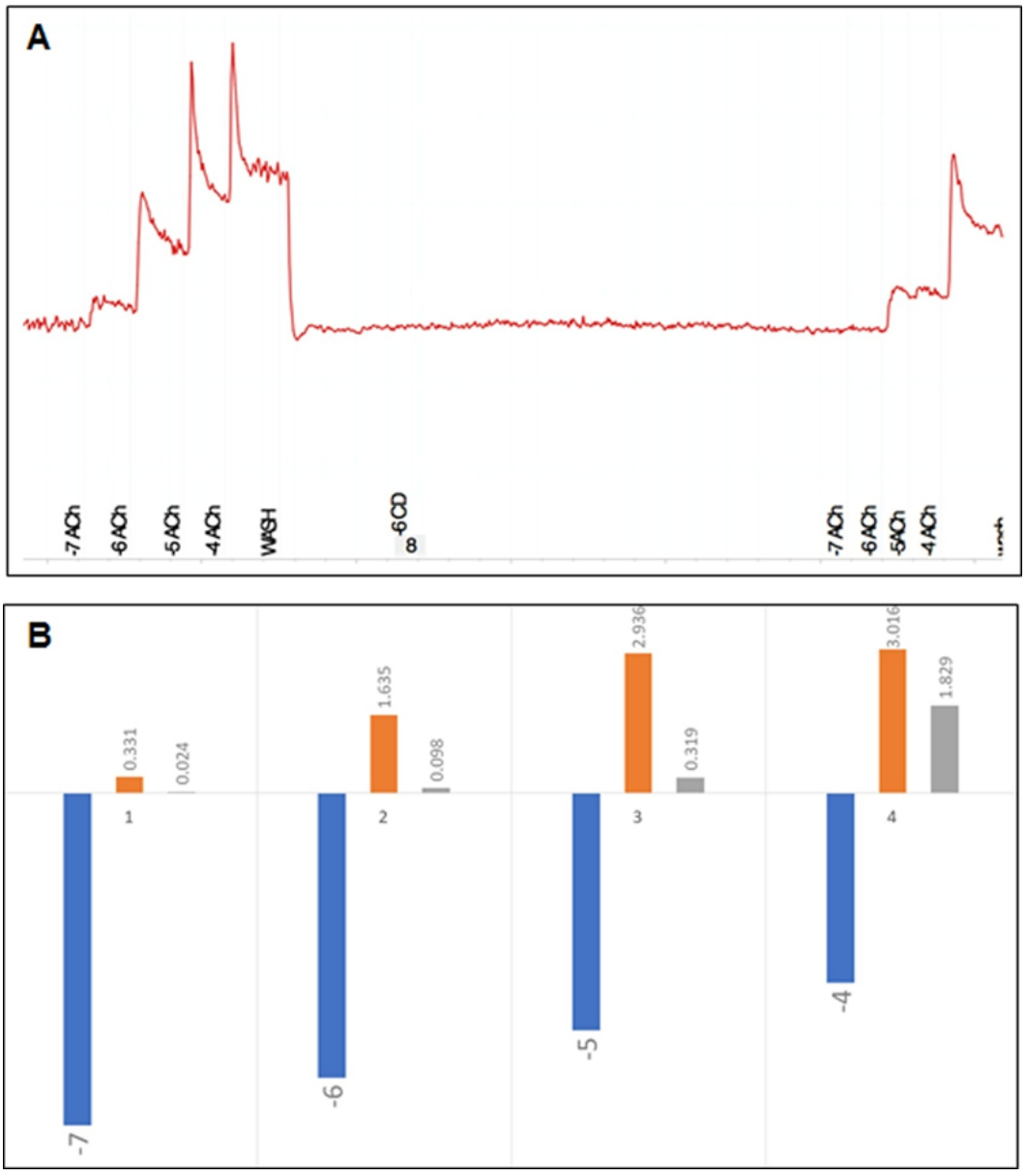

Fig. 1. Dose response contractions of rat ileum treated with CD.A, Dose response(contraction) curve of various molar concentrations (10-7to 10-4) of acetylcholine (ACh), followed by same after incubation in 0.01 µg/ml of CD; B, Bar diagram of dose response of various concentrations (10-7to 10-4) of acetylcholine (ACh), followed by same after incubation in 0.01 µg/ml of CD.

of the acetylcholine, plotted as control against different doses of CD (Fig. 2), it showed a significant variation in the Log EC_{50} values (depicted a sigmoidal dose-response± standard error).

In vivo studies in Wistar rats

The results of this study showed that CD produced statistically significant (*P<0.05*). The anti-diarrheal effect of CD was significant at the dose rate of 125mg/Kg b.wt. and 250mg/Kg b.wt. among them-selves as well as compared to the negative control during each of the hourly recording in dose dependent manner. The effect of the maximum dose (500 mg/Kg b.w.) under study, recorded antidiarrheal action were as good as the effect of loperamide at prescribed dose in our study which can be vouched in Table II.

Table II: Reduction of diarrheal droppings by different doses of CD, Standard drug (positivecontrol) and control (negative control). [n=6 in each group]

Groups	Group 1 (Negative control)	Group 2 (Positive control)	Group 3 (test)	Group 4 (test)	Group 5 (test)
Dose perKg b.wt.	CMC (0.1%) 1ml/100gb.wt.	Loperamide 2.5 mg	CD 125 mg	CD 250 mg	CD 500 mg
1 hour	10.32±0.30Da	0.67±0.13Aa	8.47±0.36Ca	5.03±0.22Ba	0.46±0.07Aa
2 hours	10.83±0.32Da	0.66±0.06Aa	8.84±0.31Ca	5.01±0.27Ba	0.52±0.05Aa
3 hours	11.30±0.23Da	0.57±0.14Aa	8.94±0.28Ca	4.94±0.23Ba	0.47±0.04Aa
4 hours	11.16±0.18Da	0.53±0.10Aa	8.66±0.32Ca	4.92±0.17Ba	0.58±0.06Aa
5 hours	11.00±0.40Da	0.53±0.05Aa	8.31±0.18Ca	5.01±0.20Ba	0.48±0.06Aa
Mean	10.92±0.13	0.59±0.04	8.64±0.13	4.98±0.09	0.50±0.02
Inhibition of Defecation (%)	0	94.552	20.804	54.324	95.40

Mean values in a column bearing different small alphabets *i.e.* a, b, c differs significantly (*P<0.05*) when compared vertically Mean values in a row bearing different capital alphabets *i.e.* A,B,C,D differs significantly (*P<0.05*) when compared horizontally

Antimicrobial studies

Further, to test whether the antidiarrheal effect of the hydroalcoholic extract of *Cordia dichotoma* leaves was due only to the reduction in the motility of the intestine or in addition, due to its effect on the diarrhea causing organisms, the hydroalcoholic extract of *Cordia dichotoma* leaves was subjected to *in-vitro* study by disc diffusion method for examining its antimicrobial effect on Gram positive (*S. aureus*) and Gram negative (*E. coli*) organisms. It was found out that the CD at various doses (250 µg, 500 µg and 1000 µg) showed anti-bacterial activity against *E. coli* (Table III) and *S. aureus* (Table IV). The antibacterial activity of CD was found greater against *E. coli* and the results were comparable to standard antibiotics used for the experiment. The dose of CD in the context of *E. coli*, at 500 µg and 1000 µg exhibited good antibacterial effects as compared to ampicillin 10 µg, chloramphenicol 30µg, gentamicin10µg and enrofloxacin 10µg (positive controls). Moreover, the results in context to *S. aureus* were not as promising, however, CD exhibited milder antibacterial effects, might help deal with the diarrhea due to bacterial infections.

Cordia dichotoma G. Forst is a versatile deciduous plant found in the tropical and sub-tropical regions of the world and India alike and its ethnobotanical studies conducted by various researchers worldwide suggest its use in the management of diarrhea (Sakkir *et al.*,2012). In the ethnobotanical studies (Lo and Duarte, 2011), it has been found that the plants belonging to Boraginaceae such as *C. myxa* (seeds), *C. rothii* (fruit pulp), *C. americana* (stem), and *C. retusa* (leaves), are used in the traditional treatment of diarrhea. Moreover, several *in-vitro* aspect *viz.* cytotoxic biological antioxidant, and anti-inflammatory (Alhadi *et al.*, 2015), antibacterial (Alhadi *et al.*, 2015; Isa, 2015) activities have been reported. Phytochemical screening test, reports on *C. dichotoma* and other plants of Boraginaceae, has claimed for their anti-diarrheal activity in animals.

Current investigation showed that CD diminished the acetylcholine-induced contractions in the ileum of the rats. The mechanism of anti-motility activity of the crude extract of *Cordia dichotoma* (CD) may be involving inhibition of cholinergic and serotonergic activity on the small intestine. Albeit, it has been reported earlier that 5-HT-induced contraction of circular muscles is blocked *in toto* by anticholinergic

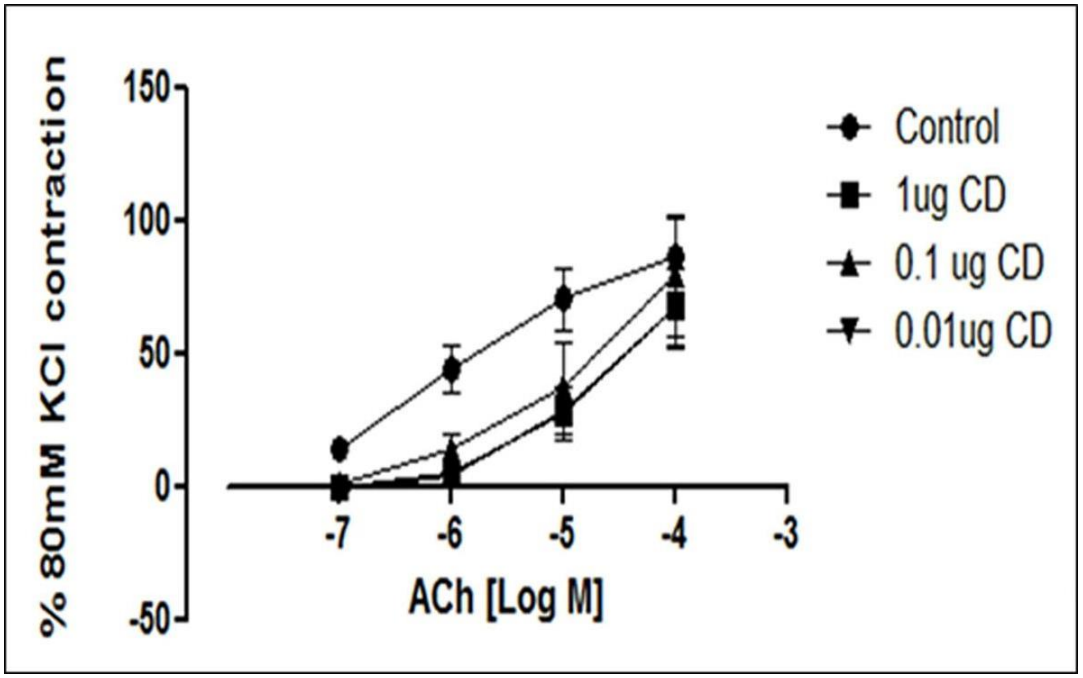

Fig. 2. Effect of various molar concentration of acetylcholine (ACh) (10-7to 10-4) followed by same after incubation in different concentration (0.01, 0.1 and 1µg/ml) of CD on contraction shown by isolated rat ileum.

drugs *viz.* atropine and hexamethonium among animals, demonstrating that 5-HT stimulates contractions of intestine via the release of ACh (Hansen, 2003) Nevertheless, we did not investigate the role of different mechanistic pathway which is the limitation of the study, it has been demonstrated that serotonin is released into the blood, post-prandial. The gut motility and bowl transit in animals has been found to be mediated via changes in pressure across the gut wall and noxious stimuli because of activation of various serotonergic receptors like 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4, by endogenous serotonin. Therefore, it is speculated with the claim that anti-serotonin activity may be the mechanism by which the herbal extract diminished intestinal motility significantly (Bardal *et al*., 2010). The herbal extract might be inhibiting excitation of myenteric and mucosal plexuses like atropine and thereby, causing reduction in intestinal motility and secretion. At this juncture it can be stated safely that the antimotility effect of CD might aid to antidiarrheal property. However, amid paucity of work, this study can provide direction for future research endeavors to isolate the active ingredient and develop novel antidiarrheal drug from *C. dichotoma* leaves, for their effect on motility of the intestines suggesting their antidiarrheal effect.

Present study has evaluated the antidiarrheal effect of hydroalcoholic extract of *Cordia dichotoma* leaf against castor oil-induced diarrhea in rats of either sex and recorded a significant decline in several diarrheic defecations. The reduction in the number of diarrheic drops has been recorded earlier in the groups of mice that received 200 mg/Kg and400mg/Kg of the extract as compared to the negative controls. In addition, they revealed significant reduction in the inhibition of intestinal fluid accumulation at the different doses of the extract (Asrie *et al*., 2016).

Although, the crude extract (CD) has been found to reduce castor oil induced diarrheal episodes, mechanism of its activity is uncertain at this juncture. It has been suggested that castor oil is hydrolyzed under the action of lipases into glycerol and ricinoleic acid, which is least absorbed from intestine. Ricinolic acid reduces absorption of water and electrolytes and stimulates the peristalsis owing to the secretion of fluid and electrolytes. The antidiarrheal effect of CD, can be proposed as antagonism

Table III: Zone of Inhibition shown by different doses of CD compared to standard drugs in case of *Escherichia coli.*

E. coli	CD 250µg	CD 500µg	CD 1000µg	Amp 10µg	CHL 30µg	Gen 10µg	ENR 10µg
Dish1	13	17	21	17	18	15	16
Dish2	14	19	22	18	19	16	18
Dish3	13	18	21	16	19	16	17
Mean	13.33	18.00	21.33	17.00	18.67	15.67	17.00
SD	0.47	0.82	0.47	0.82	0.47	0.47	0.82

CD: *Cordia dichotoma* hydroalcoholic leaves extract; Amp: Ampicillin; CHL: Chloramphenicol; Gen: Gentamicin; ENR: Enrofloxacin.

Table IV : Zone of Inhibition shown by different extracts of *Cordia dichotoma* compared to standard drugs in case of *Staphylococcus aureus*

Staph	CD 250µg	CD 500µg	CD 1000µg	Amp 10µg	CHL 30µg	Gen 10µg	ENR 10µg
Dish1	9	11	13	14	17	15	14
Dish2	8	12	12	15	18	16	14
Dish3	10	11	13	15	18	15	15
Mean	9	11.33	12.67	14.67	17.67	15.33	14.33
SD	0.82	0.47	0.47	0.47	0.47	0.47	0.47

CD: *Cordia dichotoma* hydroalcoholic leaves extract; Amp: Ampicillin; CHL: Chloramphenicol; Gen: Gentamicin; ENR: Enrofloxacin.

to the mechanism of castor oil, that leads to induction of diarrhea. The precise mechanism of action of this common laxative remains elusive, partly because of its multiple effects on the gut. It inhibits intestinal Na^+-K^+-ATPase activity (Tagne *et al*., 2019), interferes with oxidative metabolism and has effects on adenylate cyclase or mucosal adenosine 3',5'-cyclic monophosphate (cyclic AMP) content (McDonald *et al*., 2019). Castor oil is cytotoxic to intestinal epithelial cells and causes histological abnormalities with enhanced mucosal permeability. These effects may be related to the release of eicosanoids and platelet activating factor (PAF) by the intestinal mucosa (Pinto *et al*.,1992).

The antidiarrheal activity of the CD could be mediated by counter activity to the mechanisms that mediate induction of diarrhea by castor oil, *i.e.,* the extract may facilitate water and electrolyte absorption to a greater degree or reduce the oozing of fluid and electrolytes. This may be the mechanism that enables the extract (CD) to diminish the fluid overload and the diarrheal episodes in a dose-dependent manner. In addition, the extract might be influencing and interfering peristalsis in the colon, which may be a mean to delay passage of fecal matter via GIT. This antimotility activity through the GIT further buy time and therefore facilitate greater absorption of fluid, lets the stool dry through the GIT. Loperamide has this mechanism for its antidiarrheal effect.

Antimicrobial studies have been conducted in the recent past by various researchers at various locations. It has been observed that mucilage extract of *Cordia myxa* fruits has inhib-

itory effect on Gram negative bacteria including *E. coli* and *Klebsiella pneumoniae* (Jasiem *et al*., 2016). It has been found out that leaf extract of *Cordia myxa* exhibited highest inhibition in case of *Staphylococcus aureus* and then *E. coli* (Al-Snafi, 2016), whereas in current study, the effect of hydroalcoholic extract of *Cordia dichotoma* leaves was more in case of *E. coli* compared to *S. aureus*. It has been established that the extract of *Cordia dichotoma* leaves was more effective against Gram negative organisms than against Gram positive organisms and that the methicillin resistant *Staphylococcus aureus* (MRSA) was susceptible to the fruit pulp extract of *Cordia dichotoma* (Konka *et al*., 2014).

It has been demonstrated that various phytochemicals in *Cordia myxa* leaf extract i.e. flavonoids, saponins and tannins are active against pathogenic microorganisms *S. aureus* and then *E. coli* (Pandey *et al*.,2014). The effect of methanol extract of *Cordia dichotoma* Forst.

F. bark has been studied against various microorganisms like *E. coli, K. pneumoniae, P. mirabilis, Ps. Aeruginosa, S. aureus* and *P. vulgaris* at various doses and found to be more effective in case of *E. coli, K. pneumoniae, P. mirabilis* and least effective against *Ps. aeruginosa, S. aureus* (Isa, 2015). The antimicrobial activity of *Cordia dichotoma* F. leaves extract has been demonstrated against pathogenic microorganisms such as *E. coli* and *S. aureus* and against *Candida albicans* by disc diffusion method (Mulanietal.,2013). A remarkable inhibition zone of bacterial growth and fungal growth compared to standard drugs has been noticed by using methanol and butanol extracts of *Cordia dichotoma* bark (Nariya *et al*., 2012). The leaves of *Cordia dichotoma* has been shown to exhibit significant zone of inhibition against both Gram negative and Gram-positive bacteria (Sharker *et al*., 2009). The antibacterial activity of *Cordia dichotoma* leaves has been studied using two different methods i.e., disc diffusion method and well diffusion method against *E. coli* and concluded that antibacterial activity was due to flavonoid constituent (Mahour *et al*., 2008). Several bioactive molecules such as gallic acid, syringic acid, *p*-coumarin acid, caffeic acid, *p*-hydroxy benzoic acid, ferulic acid, and vanillic acid have been reported in *Cordia dichotoma* (Raina *et al*. 2022). Among these phytochemicals, caffeic acid, gallic acid, and ellagic acid have been found to help in diarrhoea-related symptoms (Dong *et al*., 2020)

Summary

It can be concluded that crude hydroalcoholic extract of *Cordia dichotoma* leaves is effective in reducing intestinal motility, preventing castor oil-induced diarrhea, and has good activity against some diarrhea causing microorganisms as well. This finding suggests that CD can be a composite treatment of diarrhea package. Therefore, the common practice of feeding leaves of *Cordia dichotoma* as a measure to tackle diarrhea in the livestock by animal owner of Jammu region can be validated. Further studies may be directed towards the extraction of the active principles responsible for the anti-diarrheal effect.

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