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The pharmacological basis of *Cuscuta reflexa* whole plant as an antiemetic agent in pigeons

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ABSTRACT

Cuscuta reflexa has been traditionally used as an antiemetic. Additionally, it has been used in various herbal formulations for the treatment of emesis. So far, there is no scientific evidence of the plant extract as antiemetic. Therefore, this study was intended to assess the antiemetic activity of Juice (JCR), aqueous (CRAE) and methanolic extract (CRME) of *C. reflexa* in pigeons. Emesis was induced through GIT irritants like ampicillin (300 mg/kg, IM), copper sulphate (100 mg/kg, PO), conc. sodium chloride solution (1600 mg/kg, PO) and cisplatin (5-HT_3 receptor stimulator) (6 mg/kg, IM). Dimenhydrinate acted as a positive control (2 mg/kg; IM). JCR [(1 ml/kg (1 %) and 1 ml/kg (2 %)], CRAE, and CRME were administered intramuscularly at different doses (50, 100 and 200 mg/kg) to each pigeon ($n = 6$). In each group, calculation of total number of jerks & vomiting episodes, and vomiting-weight was carried out to evaluate its antiemetic activity. The JCR exhibited a significant ($p < 0.05$) antiemetic impact on both the frequency and onset of emesis at 1 ml/kg (2 %) against various emesis mediator, except sodium chloride. Similarly, CRAE and CRME elicited marked dose dependent inhibition both on onset and frequency of emesis with highly significant ($p < 0.001$) effect at 200 mg/kg. The study reflects that juice, aqueous and methanolic extract of *C. reflexa* have significant antiemetic potential and possess pharmacological active constituent(s) that interfered with the emetic mediators by acting through GIT irritation and 5-HT_3 receptor stimulations. Results of this study provide a scientific background to its traditional antiemetic uses.

1. Introduction

Natural products based preparations play a key role in human and veterinary medicines, agriculture, food and even cosmetics [1]. Herbal remedies are used in developed and undeveloped countries of the world for the treatment of several human disorders. Moreover, natural products are known to display a significant role in curing of chronic disorders [2]. It is estimated by World Health Organization (WHO) that 90 % residents of developing countries are mainly practicing and utilizing plant-based medications [3]. The need of these herbal medicines is growing day by day because of the identification of plants, as a medicinal source is getting clear and knowledgeable, currently experiencing a revival in Western society [4]. These medicinal plants are potential source as a remedy for various chronic and acute disorders such as constipation, emesis, diarrhea etc.

Cuscuta reflexa is a parasitic perennial herb that belongs to family

Abbreviations: JCR, juice of *Cuscuta reflexa*; CRAE, *Cuscuta reflexa* aqueous extract; CRME, *Cuscuta reflexa* methanolic extract; ANOVA, analysis of variance; IM, intra muscular; PI, percent inhibition.

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Convolvulaceae and is present around the globe particularly in South Asian countries like Pakistan, Nepal, India, and Bangladesh [5]. Out of total 145 species in present in Genus *Cuscuta* L., 14 species are available in Pakistan [6]. Various pharmacological studies have revealed the used of this herb as an antihistaminic, anticholinergic, anti-hypertensive [7], antibacterial, antioxidant [8], anthelmintic [9], and anti-inflammatory [10] agents for treatment of several maladies including alopecia [11], HIV [12], diabetes [13], epilepsy [14], CNS depression, pain [15], tumor [16] and urinary infections [17].

Furthermore, phytochemistry of *C. reflexa* elucidates that it constitutes of carotenoids, sitosterol, caffeic acid, mannitol, flavonol, dulcitol, quercetin, tannins, lutein, lycopene, kaempferol, coccinoside B, kaempferol-3-O-glucoside, stigmasterol, cuscutamine, quinic acid, 6,7-dimethoxy coumarin, cuscutalin, isorhamnetin-3-O-neohesperidoside, myricetin, abscisic acid, reflexin, bergenin, and some other polyphenols like 3,4-O-dicaffeoyl quinic acid, leuteolin, quercetin-3-O-glucoside, apigenin-7-β-rutinoside, amarbelin, coccinoside, alpha-amyrin, kaempferol-3-O-*α*-rhamnoside, β-setosterol, *α*-cryptoxanthin, cuscutin, cuscutoside-A, and apigenin-7-O-glucoside [5,12,18].

In Pakistan, this herb is being practiced in traditional medications to deal with vomiting and nausea conditions without any scientific evidence. Therefore, keeping in view its phyto-chemistry and current usage in herbal medications, this study was carried out to validate antiemetic effect of juice, aqueous, and alcoholic extracts from *C. reflexa* whole plant against various mediators of emesis.

2. Materials and methods

2.1. Plant material

In March 2016, the availability of *C. reflexa* (whole plant) was ensured form Takht Bhai (Mardan-Khyber Pukhtunkhwa) Pakistan from *Eucalyptus globulus* as a host plant. Identification and confirmation was done by a taxonomist at Department of Botany, Abdul Wali Khan University Mardan (AWKUM), Khyber Pukhtunkhwa (KPK)-Pakistan. For further reference purposes, a voucher number *i.e.* AWKUM. BOT. 189 (1) 2 was placed in herbarium of Abdul Wali Khan University Mardan.

2.2. Drugs and chemicals

For this experimentation, distilled water was procured from Unisa Pharmaceuticals (PVT) LTD, Pakistan. While, Ampicillin was made available from Bosch Pharma (PVT) LTD, Pakistan. Sodium chloride and Copper sulphate were purchased from Unisa Pharmaceuticals (PVT) LTD, Pakistan. Other chemicals used in this study were as Dimenhydrinate (Searle Pakistan (PVT) LTD), Methanol (Master chemical supplier Pakistan), Ondansetron (Pharmedic PVT Limited, Pakistan), and Cisplatin which was purchased from Pfizer Laboratories LTD, Pakistan.

2.3. Experimental animals

In this study, Healthy Pigeons weighing 240−380 g of either sex were caged and were provided with maintained experimental conditions of temperature (25−30 °C) and light-dark cycles (12−12 h). Availability of fresh water and free access to locally formulated food (Millet + Wheat grains) were ensured throughout the experimental procedure [19]. Ethical Review Committee (ERC), Department of Pharmacy, Abdul Wali Khan University Mardan, approved the experimental considerations of the study. Moreover, this research fulfilled the all the international standards and principles set up for Care and Use of animals in Laboratory research experiments [20].

2.4. Preparation of juice and extracts

2.4.1. Juice of Cuscuta reflexa (JCR)

For the preparation of Juice samples (JCR) of *Cuscuta reflexa*, whole plant (3.2 Kg) was washed thoroughly for the removal dust, dirt, and debris particles. Afterwards, plant was size reduced by using knife followed by subjection to squeezing mill with a high force for the collection of resultant juice samples. The fresh juice was used for biological effect.

2.4.2. Aqueous extract of Cuscuta reflexa (CRAE)

Whole plant (5 Kg) was cleaned from dust, dirt, debris, and damaged portions were removed manually. Plant was dried under shade at 30–35 °C and was powdered using commercial grinder. Powdered plant (3.2 kg) was then macerated with water (6 L) at room temperature for 15 days with occasional stirring. Afterwards, they were filtered using muslin cloth for the separation of insoluble plant material and soluble aqueous residues. Soluble aqueous residues were further filtered using filter paper (Whatman No. 1). This process was repeated second time with addition of 3 liters water to the insoluble plant material separated from the first maceration process. The resultant soluble aqueous residue from second maceration process was pooled up with the soluble residues of first step. The final filtrate was placed at room temperature (under-shade) for 40 days, to ensure maximum removal of moisture. Following this step, a solid mass of crude aqueous extract (28 g) was obtained which was further powdered to ensure easy dissolution.

2.4.3. Methanolic extract of Cuscuta reflexa (CRME)

Methanolic extract was obtained from Herbion Pakistan Pharmaceutical (PVT) Limited, a leading herbal products preparation industry in Pakistan.

2.5. Experimental design

2.5.1. Grouping and dosing for antiemetic model

Pigeons were divided into 10 different groups (n = 6); group 01 (negative control) was provided with distilled water (10 ml/kg; IM), group 02 (positive control) was given Dimenhydrinate (2 mg/kg, IM), group 03 and 04 were treated with varied concentration of JCR *i.e.* 1 ml/kg (1 %) and 1 ml/kg (2 %) through IM route respectively. While, group 05, 06, and 07 received various doses of CRAE *i.e.* 50, 100, and 200 mg/kg (IM), respectively. Similarly, groups 08, 09, and 10 were administrated with CRME at varied concentrations (50, 100, and 200 mg/kg) via IM route. For the induction of emesis, pigeons were subjected to emetic chemicals post thirty minutes administration of tested samples [20]. In this study, response of pigeons without oral expulsion and with oral expulsion were classified as jerk and vomiting, correspondingly. For next 12 h, time to first jerk & vomit, latency to first jerk & vomit time, number of jerks & vomits, and vomit weight for each vomit was noted by following the defined protocols [20].

2.5.2. Models for induction of emesis

2.5.2.1. Ampicillin induced emesis model. The experiment was performed by following the already described method of Bass et al. [21]. In this model, emesis was induced in pigeons by administration of ampicillin (IM: 300 mg/kg) post 30 min of treatment with Distilled water, standard drug, JCR, CRAE and CRME.

2.5.2.2. Copper sulphate induced emesis model. Purposely, pigeons were divided into groups depending upon their treatment with distilled water, standard drug, JCR, CRAE and CRME. Induction of emesis in this model was achieved by oral subjection of Copper sulphate (100 mg/kg) to each test animals, 30 min after application of above mentioned treatments [22].

2.5.2.3. Sodium chloride induced emesis model. This experiment was performed with slight modification in previously described method of Casavant and Fitch [23]. All the pigeons were initially treated with distilled water, standard drug, JCR, CRAE and CRME. For this model, 30

min after the application of above mentioned treatments, induction of emesis in these treated pigeons were achieved by orally subjecting them to sodium chloride (1600 mg/kg).

2.5.2.4. Cisplatin induced emesis model. At the start of the trial, pigeons used in this model were treated with Distilled water, standard drug, JCR, CRAE and CRME. Then, thirty minutes after treating them with above mentioned subjects, Cisplatin (IM: 6 mg/kg) was administered to these pigeons for induction of emesis [19]. For all the above mentioned models, experimental pigeons were compared with the negative control group with reference to number & consistency of jerks and emesis episodes, separately.

2.6. Statistical analysis

Results were represented as mean ± standard error of mean (SEM). To assess the level of significance ($p < 0.05$) of *Cuscuta reflexa* whole plant against emesis induced in pigeons using various models, Analysis of variance (One-way ANOVA) followed by Dunnett's test was used.

3. Results

3.1. Effects of C. reflexa plant in ampicillin-induced emesis model

Various extracts of *C. reflexa* plant demonstrated a dose-dependent antiemetic potential in pigeon model which were pretreated with distilled water, standard drug, and various types of tested plant materials (JCR, CRAE, and CRME) and then were subjected to ampicillin (300 mg/kg, IM) for initiation of emesis. JCR at the dose of 1 ml/kg (1 %) was almost insignificant (Table 1), while at dose 1 ml/kg (2 %), JCR exhibited momentous ($p < 0.05$) results in all the observed examined parameters as shown in Table 1. Further, CRAE at the dose of 100 and 200 mg/kg showed statistically noteworthy results regarding first vomiting time, latency to first vomiting time, and number of vomiting episodes (Table 1). Similarly, CRME (100 and 200 mg/kg) demonstrated momentous effect regarding first vomiting time, latency to first vomiting time (Table 1), and vomiting weight (Fig. 1).

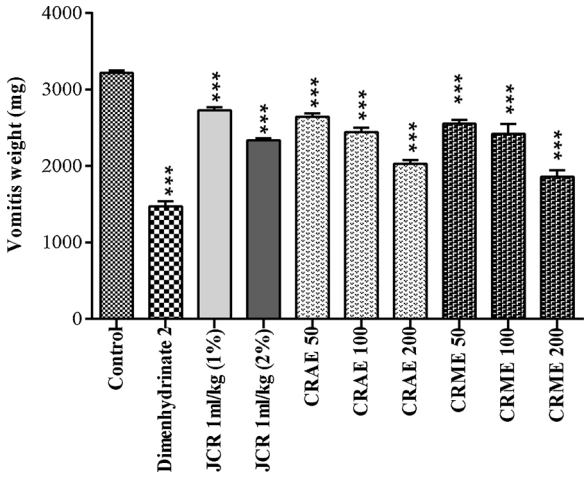

Fig. 1. Bar diagram indicating dose dependent effect of JCR, CRAE and CRME against ampicillin induced emesis in pigeons. Bars are presented as mean ± SEM for groups (n = 6). One-way ANOVA followed by Dunnett's test was applied for data analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus negative group (Asterisks representing statistically significant values from control).

3.2. Effects of C. reflexa plant in copper sulphate-induced emesis model

All tested samples (JCR, CRAE, and CRME) of *C. reflexa* plant showed a concentration-dependent potential in copper sulphate-induced emetic pigeons. JCR [(1 ml/kg: (1 %)] showed non-significant effect on all the experimented traits (Table 2). However, at higher dose *i.e.* 1 ml/kg (2 %), JCR significantly attenuated the first vomiting time, latency to first vomiting time, & number of vomiting episodes ($p < 0.05$). Similarly, at this dose, JCR produced considerably ($p < 0.001$) less weight of vomiting as compared to the negative control (Fig. 2). Out of all the examined doses (50−200 mg/kg) of CRAE, just 200 mg /kg showed significant ($p < 0.01$) results when compared to the control group. Whereas, CRAE at the dose of 50 mg/kg, 100 mg/kg, and 200 mg/kg resulted in significantly ($p < 0.001$) less vomiting weight in comparison to negative control group (Fig. 2). Similarly, in case of CRME

Table 1
Effect of JCR, CRAE and CRME on ampicillin induced emesis in pigeons.

Group (mg/kg, IM)	1st vomiting time (min)	Latency to 1st vomiting time (min)	Number of vomiting episodes (8 h)	Number of jerks (8 h)
Control	10.13 ± 2.86	5.12 ± 1.28	9.12 ± 2.75	18.19 ± 3.80
Dimenhydrinate 2	25.24 ± 1.15***	14.56 ± 0.60***	3.53 ± 0.69***	7.51 ± 1.15***
JCR 1 ml/kg (1 %)	12.67 ± 4.72^{ns}	6.41 ± 3.15^{ns}	8.28 ± 5.14^{ns}	15.95 ± 4.54^{ns}
JCR 1 ml/kg (2 %)	14.51 ± 6.30*	7.71 ± 3.02*	7.31 ± 2.36*	13.64 ± 2.79**
CRAE 50	12.58 ± 4.40^{ns}	6.92 ± 3.08^{ns}	8.01 ± 3.89^{ns}	14.87 ± 2.65^{ns}
CRAE 100	15.99 ± 3.65*	8.56 ± 1.21*	6.43 ± 2.14*	12.81 ± 4.89**
CRAE200	21.75 ± 2.85**	11.73 ± 2.85**	5.87 ± 1.53**	11.16 ± 3.11**
CRME 50	13.94 ± 3.34 ^{ns}	7.11 ± 2.14^{ns}	7.59 ± 3.95^{ns}	12.94 ± 4.69^{ns}
CRME 100	16.93 ± 1.60*	9.77 ± 2.34*	6.07 ± 2.28**	11.09 ± 2.95**
CRME 200	22.83 ± 1.92***	12.86 ± 1.66***	5.13 ± 1.59**	10.35 ± 2.29***

Data are presented as mean ± standard error of the mean for groups (n = 6). A One-way ANOVA followed by Dunnett's test was applied for data analysis. *p < 0.05, **p < 0.01, ***p < 0.001 versus negative group (Asterisks representing statistically significant values from control).

Table 2
Effect of JCR, CRAE and CRME on copper sulphate induced emesis in pigeons.

Group (mg/kg, IM)	1st vomiting time (min)	Latency to 1st vomiting time (min)	Number of vomiting episodes (8 h)	Number of jerks (8 h)
Control	12.32 ± 3.60	2.19 ± 0.57	9.01 ± 2.57	11.11 ± 2.57
Dimenhydrinate 2	28.59 ± 3.15***	9.84 ± 1.09***	4.34 ± 1.86**	5.41 ± 1.86***
JCR 1 ml/kg (1 %)	14.39 ± 3.46^{ns}	3.41 ± 2.15^{ns}	8.08 ± 3.15^{ns}	10.54 ± 2.73^{ns}
JCR 1 ml/kg (2 %)	17.61 ± 2.30*	5.49 ± 2.73*	7.53 ± 2.50*	8.27 ± 2.15*
CRAE 50	15.37 ± 2.34^{ns}	4.57 ± 2.21^{ns}	8.07 ± 2.01^{ns}	9.82 ± 2.02^{ns}
CRAE 100	17.84 ± 4.66^{ns}	5.86 ± 2.47^{ns}	6.71 ± 3.14*	9.11 ± 1.21*
CRAE200	22.23 ± 3.86**	7.61 ± 2.66***	6.17 ± 1.40**	7.59 ± 1.47**
CRME 50	16.37 ± 4.47^{ns}	4.06 ± 1.34^{ns}	7.54 ± 0.96^{ns}	8.67 ± 1.08^{ns}
CRME 100	19.83 ± 4.63*	6.82 ± 2.59*	6.10 ± 1.21*	7.41 ± 1.28*
CRME 200	24.74 ± 5.92**	7.83 ± 1.79**	5.61 ± 2.66**	6.05 ± 1.59**

Data are presented as mean ± standard error of the mean for groups (n = 6). A One way ANOVA followed by Dunnett's test was applied for data analysis.*p < 0.05, **p < 0.01, ***p < 0.001 versus negative group (Asterisks representing statistically significant values from control).

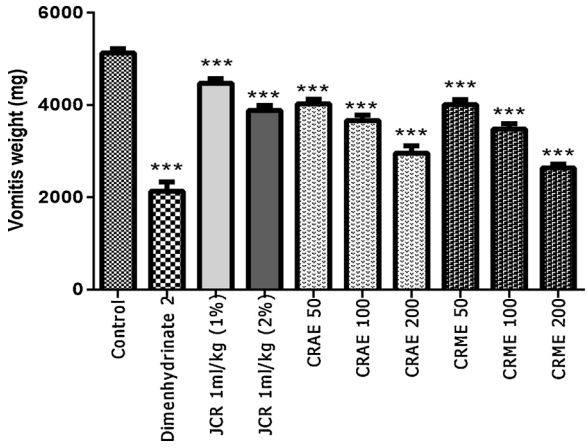

Fig. 2. Bar diagram indicating dose dependent effect of JCR, CRAE and CRME against copper sulphate induced emesis in pigeons. Bars are presented as mean ± SEM for groups (n = 6). A One way ANOVA followed by Dunnett's test was applied for data analysis.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus negative group (Asterisks representing statistically significant values from control).

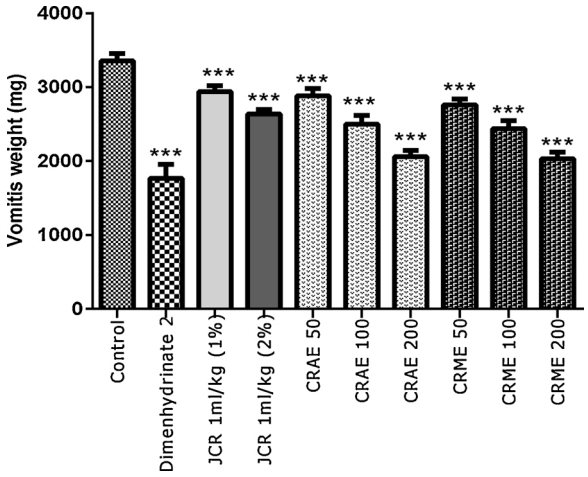

Fig. 3. Bar diagram indicating dose dependent effect of CRAE and CRME against concentrated solution of sodium chloride induced emesis in pigeons. Bars are presented as mean ± SEM for groups (n = 6). A One way ANOVA followed by Dunnett's test was applied for data analysis.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus negative group (Asterisks representing statistically significant values from control).

administration (200 mg/kg) exhibited highly significant ($p < 0.01$) results relative to first vomiting time, latency to first vomit time, number of vomit episodes, number of jerks and produced statistically noteworthy ($p < 0.001$) less weight of vomiting (Fig. 2).

3.3. Effect of C. reflexa plant in sodium chloride (NaCl)-induced emesis

Pretreatment of pigeons with various doses of JCR, CRAE and CRME demonstrated a dose-dependent antiemetic effect against NaCl-induced emesis. Pre-treatment of 1 % JCR (1 ml/kg) was non-significant as clear from Table 3, but highly significant ($p < 0.001$) in terms of reduced vomiting weight. On the other hand, JCR 1 ml/kg (2 %) showed significant ($p < 0.05$) effects on vomiting episodes and number of jerks, while results regarding weight of vomiting were highly momentous ($p < 0.001$) (Fig. 3). Pre-treatment of CRAE (100 and 200 mg/kg) showed significant ($p < 0.05$) results regarding all the examined parameters.

Table 3
Effect of JCR, CRAE and CRME on sodium chloride induced emesis in pigeons.

Group (mg/kg, IM)	1st vomiting time (min)	Latency to 1st vomiting time (min)	Number of vomiting episodes (mean)	Number of jerks (mean)
Control	12.12 ± 3.57	7.52 ± 1.57	7.11 ± 2.28	11.34 ± 2.57
Dimenhydrinate 2	22.42 ± 3.15***	15.71 ± 2.86***	3.32 ± 0.57***	3.62 ± 0.86***
JCR 1 ml/kg (1 %)	13.41 ± 2.02^{ns}	9.42 ± 2.16^{ns}	6.41 ± 1.86^{ns}	9.42 ± 1.03^{ns}
JCR 1 ml/kg (2 %)	15.36 ± 2.30^{ns}	11.14 ± 2.30^{ns}	5.63 ± 1.15*	7.41 ± 2.44*
CRAE 50	14.44 ± 2.40^{ns}	10.37 ± 1.96^{ns}	6.57 ± 3.70^{ns}	10.57 ± 1.90^{ns}
CRAE 100	17.11 ± 3.72*	12.15 ± 2.28*	5.67 ± 2.08*	8.55 ± 1.21*
CRAE200	19.17 ± 4.98***	13.59 ± 3.66**	4.73 ± 1.21**	7.62 ± 1.55**
CRME 50	15.29 ± 3.53^{ns}	10.27 ± 2.14^{ns}	6.03 ± 1.90^{ns}	9.65 ± 0.95 ^{ns}
CRME 100	18.57 ± 2.79*	12.89 ± 2.47*	5.11 ± 1.14*	7.64 ± 1.47*
CRME 200	21.45 ± 2.24**	14.01 ± 4.73**	3.87 ± 1.40***	6.37 ± 1.59***

Data are presented as mean ± standard error of the mean for groups (n = 6). A One way ANOVA followed by Dunnett's test was applied for data analysis.*p < 0.05, **p < 0.01, ***p < 0.001 versus negative group (Asterisks representing statistically significant values from control).

Likewise, CRAE (100 and 200 mg/kg) showed significantly ($p < 0.001$) less weight of vomiting as compared to those observed in negative control group (Fig. 3). Similarly, CRME (100 and 200 mg/kg) caused significant ($p < 0.05$) effects on all the examined traits as shown in Table 3. Compared to control (negative) group it was very interesting that CRME at the dose of 100 and 200 mg/kg exhibited considerably ($p < 0.001$) less vomiting weight (Fig. 3).

3.4. Effect of C. reflexa plant in cisplatin induced emesis

Pretreatment of pigeons with JCR, CRAE and CRME at various doses showed significant antiemetic effect in case of cisplatin induced emesis

Table 4
Effect of JCR, CRAE and CRME on cisplatin induced emesis in pigeons.

Group (mg/kg, IM)	1st vomiting time (min)	Latency to 1st vomiting time (min)	Number of vomiting episodes (mean)	Number of jerks (mean)
Control	71.23 ± 4.60	6.16 ± 2.86	56.19 ± 4.73	41.45 ± 4.73
Dimenhydrinate 2	96.46 ± 5.46**	13.62 ± 3.15**	27.10 ± 2.30***	32.66 ± 3.30**
Ondasetron 0.50	121.51 ± 7.88***	15.32 ± 2.57***	22.42 ± 3.73***	19.67 ± 3.88***
JCR 1 ml/kg (1 %)	75.12 ± 5.04^{ns}	7.51 ± 1.73^{ns}	48.85 ± 4.46^{ns}	38.36 ± 4.04^{ns}
JCR 1 ml/kg (2 %)	78.92 ± 4.91*	9.90 ± 2.30*	44.55 ± 4.04*	35.58 ± 4.61*
CRAE 50	75.58 ± 5.22^{ns}	8.37 ± 1.26^{ns}	43.38 ± 4.95^{ns}	40.38 ± 3.95^{ns}
CRAE 100	79.57 ± 4.35*	10.57 ± 3.51*	40.65 ± 2.68*	36.69 ± 4.33*
CRAE200	89.67 ± 5.01***	12.24 ± 3.83**	36.47 ± 4.58***	33.57 ± 3.65**
CRME 50	76.68 ± 3.85^{ns}	8.89 ± 1.40^{ns}	44.55 ± 4.05^{ns}	40.04 ± 3.07^{ns}
CRME 100	83.34 ± 4.68*	11.27 ± 3.66*	42.58 ± 4.13**	35.58 ± 3.45**
CRME 200	91.27 ± 5.12***	12.86 ± 2.04***	33.64 ± 3.70***	33.09 ± 3.78***

Data are presented as mean ± standard error of the mean for groups (n = 6). A One way ANOVA followed by Dunnett's test was applied for data analysis.*p < 0.05, **p < 0.01, ***p < 0.001 versus negative group (Asterisks representing statistically significant values from control).

(Table 4). JCR at 1 ml/kg (2 %) revealed significant ($p < 0.05$) effect on all the experimented parameters as shown in Table 4 and significantly ($P < 0.001$) less vomiting weight was also noticed (Fig. 4). CRAE (100 and 200 mg/kg) revealed antiemetic effect as it elicited significant ($P < 0.05$) effect on 1st vomiting time, latency to 1st vomiting time, number of vomiting episodes and number of jerks. Further, at 200 mg/kg highly significant ($P < 0.001$) reduction in vomits weight (Fig. 4) was also noticed. CRME at 100 mg/kg produced significant ($p < 0.05$) effects that further improved at the dose of 200 mg/kg ($p < 0.001$). Regarding vomits weight CRME (100 and 200 mg/kg) showed momentously ($p < 0.001$) less weight as clear from Fig. 4.

4. Discussion

Nausea is known as feeling of being vomit while vomiting on the other hand is known as vigorous discharge of ingested materials via mouth. Swallowing of vomit is a reflexive mechanism harmonized by area specified (vomiting-center) in brain stem that is positioned in medulla oblongata [24]. Chemo-receptor trigger zone (CTZ) is an area which is responsible for receiving hormonal input and communicating with other structures of vomiting center [25]. CTZ could be triggered due to excessive swollenness of small intestines and/or stomach region, continuous movement of head (sensation of wooziness), elevated brain pressure, ingestion of poisonous material, and intensive pain [25].

Ampicillin is one of the effectual anti-biotic, which is being prescribed quite commonly throughout the world. Various clinical studies regarding the excessive usage of ampicillin may cause side effects like diarrhea, nausea, and vomiting [21]. Elevated dosage of antibiotics may activate the gastrointestinal (GI)-motor activity leading to intense tightening of GIT (gastro-intestinal-tract) eventually resulting the induction of emesis. It has been documented that atropine has inhibitory effect on gastrointestinal motor activity by suppressing the actions of acetylcholine at parasympathetic sites in smooth muscles, secretory glands, & central nervous system (CNS) [26]. Emesis induced by copper sulphate might be due to stimulation of terminals of vagus nerve visceral afferents (VA) that innervate through the stomach wall in living creatures. This excitation of GI-VA is triggered by cisplatin causing activation of 5-HT_3 receptors resulting in induction of emesis [27]. Ingestion of sodium chloride solution in concentrated form also induces emesis and act as intense gastro-intestinal irritant. Induction of emesis via concentrated sodium chloride solution are found to be linked with other metabolic conditions such as tachycardia, hyper-tension, hypernatremia, and hyperthermia [23]. In upper GIT, cisplatin-an anticancer drug-result in discharge of serotonin from ECL (enterochromaffin-like cells) resulting in the stimulation of gastrointestinal-VA through 5-HT_3 receptors eventually inducing emesis [19]. Literature have shown gastro-protective potential of various ethno-medicinal plants in experimented rat models [28].

In this research trail, pigeons were used as vomiting model [27,29,30] in which induction of emesis was achieved using ampicillin, $CuSO_4$, conc. NaCl, and Cisplatin, separately. All the experimented *C. reflexa* plant extracts (JCR, CRAE, and CRME) elucidated anti-emetic potential in a dose-dependent manner owing to their anti-histaminic and anti-cholinergic perspectives [7,31]. It has been reported that *C. reflexa* plant possess inhibitory potential on acetylcholine [5,32]. Various compounds isolated from different parts of *C. reflexa* plant like β-setosterol and stigmasterol etc. are reported to be responsible for this inhibitory activity [33]. Earlier investigations have revealed the role of steroidal constituents like corticosteroids possessing antiemetic potential in experimental subjects. Therefore, antiemetic properties of steroidal compounds present in *C. reflexa* plant are known to have antiemetic effect that might be due to blockage of dopamine receptors, increase of GABAergic transmissions, and/or antihistaminic potentials [5]. On the basis of structure similarity of different compounds of *C. reflexa* with standard antiemetic drugs, like ondansetron with cuscutamine Ondansetron a standard antiemetic drug is one of the indole

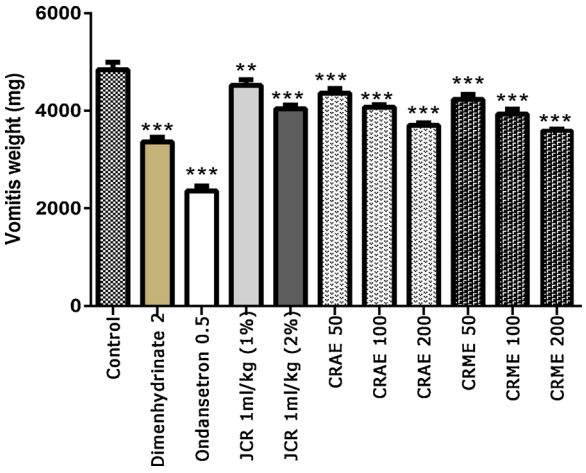

Fig. 4. Bar diagram indicating dose dependent effect of JCR, CRAE and CRME against cisplatin induced emesis in pigeons. Bars are presented as mean ± SEM for groups (n = 6). One-way ANOVA followed by Dunnett's test was applied for data analysis*$p <$ 0.05, **$p <$ 0.01, ***$p <$ 0.001 versus negative group (Asterisks representing statistically significant values from control).

derivatives and cuscutamine is also an indole containing compound. It should be tested for the antiemetic activity as mostly same structure compounds have similar action. Other member of family convolvulacea also having antiemetic potential [33]. Petroleum extract of *C. reflexa* plant prolonged the sedation induced by meprobamate, diazepam, and pentobaritone [15]. Methanolic extract of *C. reflexa* plant has been reported to elevate the concentration of 5-hydroxytryptamine (5-HT) and melatonin, which helped in promoting sleep [14]. The elucidated sedative potential of this plant might be the reason behind the significant antiemetic effect. Extract of *C. reflexa* actually blocks the histaminergic receptors (CNS) such as dimenhydrinate possessing anticholinergic, CNS depressant, and antihistaminic potentials. Various earlier investigations have illuminated the role of phytochemicals present in *C. reflexa* responsible for blockage of dopamine receptors [5]. This mentioned mode of action might be responsible for *C. reflexa's* antiemetic potential just like in case of metoclopramide and domperidone (antiemetic drugs) acting as selective antagonist of dopamine D2-receptor. Nevertheless, further research-based investigation is required to validate the anti-emetic characteristics of *C. reflexa* together with stated anti-tumor and anti-cancer potentials [16,34].

5. Conclusion

This experimental study has shown that *C. reflexa* contains pharmacologically active substances, which provided maximum protection against these emesis inducers in pigeons. This antiemetic potential is may be due to suppression of GIT motility/gastric emptying/irritation induced by various GIT irritants and inhibition of 5-HT_3 receptor stimulation caused due to induction of emesis through cisplatin. These attributes may provide rational use of this plant in emesis and thus supporting the folkloric use of this plant by traditional healers. Moreover, further detail studies on the isolation of compounds from this plant followed by their antiemetic effect, is highly suggested in order to discovery molecules of clinical utility.

Declaration of Competing Interest

The authors report no declarations of interest.

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