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Argemone mexicana extract alleviates gastrointestinal disorders by stimulating muscarinic receptors and blocking voltage-gated *L*-type calcium channels

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ABSTRACT

Objective: To investigate the pharmacological potential of *Argemone mexicana* in treating constipation and emesis by using *in vitro* and *in vivo* models.

Methods: The spasmogenic and spasmolytic effects were evaluated on isolated rabbit jejunum fragments loaded in a tissue organ bath. The response was recorded with an isotonic transducer attached with Power Lab Data Acquisition System. The laxative and antiemetic activities were assessed in BALB-c mice and poultry chicks challenged with carbamylcholine and copper sulphate stimulated emesis, respectively.

Results: The total phenolic and total flavonoids contents of the extract were (267.75 ± 5.77) mg GAE/g and (73.86 ± 6.01) mg QE/g, respectively. *Argemone mexicana* extract exerted spasmogenic effect on isolated rabbit jejunum segments with an EC_{50} value of 0.016 mg/mL, which was blocked by atropine (0.3 μM). *Argemone mexicana* extract exerted spasmolytic effect in atropine treated jejunum fragments with an EC_{50} value of 2.185 mg/mL. Furthermore, *Argemone mexicana* extract relaxed potassium (80 mM)-induced contractions (EC_{50}: 9.07 mg/mL), similar to a standard drug verapamil. The calcium channel blocker activity was confirmed by a rightward shift of concentration-response curve of calcium in the presence of *Argemone mexicana* extract (1-5 mg/mL) and verapamil (0.1-1 μM). In addition, the extract increased the distance travelled by a charcoal in the gastrointestinal tract and exhibited antiemetic effect on copper sulphate induced emesis in chicks.

Conclusions: *Argemone mexicana* shows cholinergic agonist and calcium channel blocker activities, as well as antiemetic effect. It may be used as a potential agent for treating gastrointestinal disorders.

KEYWORDS: *Argemone mexicana*; Muscarinic agonist; Calcium channel blocker; Constipation; Emesis

1. Introduction

Natural medicines have been continuously used since the beginning of human civilization and are still preferred by people due to devastating adverse effects and high cost of synthetic drugs. Natural products are unique combinations of multiple phytochemicals which are prescribed by local practitioners and herbalists or traditionally used[1]. *Argemone mexicana* (*A. mexicana*) Linn (Family: Papaveraceae) known by its vernacular name Satyanasi

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is a prickly glabrous annual herb that commonly grows throughout South Asia[2]. Several phytochemical constituents including berberine and sanguinarine have been detected in *A. mexicana*[3]. The aerial part of the plant is utilized for the treatment of malaria. It is reported that the aerial part has analgesic, spasmolytic, anti-parasitic and narcotic properties. Fresh juice of leaves is topically applied to promote the wound healing, whereas the milky seed extract is useful as folkloric remedy of cold sores, oedema, jaundice, leprosy, malarial fever, warts, wounds and skin-related diseases[4]. Likewise, the plant extract showed several pharmacological actions including antibacterial, anti-inflammatory, antiseptic, antiviral, anti-emetic, expectorant, fungicidal, hypotensive, hypoglycaemic, laxative, respiratory stimulant, spasmogenic, and vasodilator activities[5]. Smooth muscle of the gastrointestinal tract regulates the gut motility. Functional abnormality in intestinal smooth muscle results in multiple gastrointestinal problems such as spasm, cramps, constipation, diarrhoea, and emesis. The present work was aimed to investigate the therapeutic effect of *A. mexicana* on gastrointestinal problems such as constipation and emesis.

2. Materials and methods

2.1. Chemicals

All the chemical reagents were of analytical grade. Carbamylcholine chloride, atropine, verapamil, ethylenediaminetetraacetic acid, magnesium chloride ($MgCl_2$), sodium chloride (NaCl) and potassium chloride (KCl) (Sigma Chemicals MO, St Louis, USA), copper sulphate (Sigma Aldrich, Germany), Folin and Ciocalteu's Reagent (Unichem Chemicals), gallic acid, (Sino-chem, China), ethanol (RDH, Germany), quercetin, (Sigma Life Science, Germany), glucose ($C_6H_{12}O_6$), $CaCl_2$, $MgSO_4$, KH_2PO_4, NaH_2PO_4 and $NaHCO_3$ (Merck, Darmstadt, Germany), chlorpromazine (High-Noon Laboratories, Lahore, Pakistan) were purchased. Moreover, freshly prepared isotonic solutions were used in experiments.

2.2. Animals and housing conditions

The BALB-c mice (♂/♀; 25-30 g) were acquired from the Animal house of the University of Lahore, while the rabbits of the local breed (♂/♀; 1.0-1.5 kg) and chicks (♂/♀; 40-50 g) were purchased from the local animal market of Lahore. The animals were kept at optimum laboratory conditions [temperature: (25±2) ℃, humidity: (55±5)% and 12 h day/night cycles] in the animal house of the University of Lahore. The animals were given standard pellet diet and tap water *ad libitum*. They were acclimatized for 14 d and fasted overnight prior to the experiment. The experiments were performed by following the guidelines of the Commission of Laboratory Animal Resources of Life Sciences[6].

2.3. Extract preparation of A. mexicana

The fresh plant was collected from Mirpur Azad Kashmir and identified by Prof. Dr. Zaheer-ul-Deen, an expert botanist from Department of Botany, Govt College University, Lahore. The voucher number assigned to *A. mexicana* was GC.Herb.Bot. 3528 and the sample was submitted for further reference in the department herbarium. The fresh plant was shade dried, cleared from impurities, and crushed by herbal mill (Model: WF-120 Universal Pulverizer, Shanghai, China) to a coarse powder. The pulverized powder was macerated with hydro-alcoholic solvent (30:70) at 25 ℃ for 14 d with occasional manual shaking and the powder solvent ratio was 1:10. The sample was filtered *via* muslin cloth and then through Whatman's filter paper 1, and the collected filtrate was concentrated to thick semi-solid mass at 30-40 ℃ under low pressure on Rota-vapour (Model: HS-2005S, Hahnshin scientific, Korea) attached to a vacuum pump (HVP-W2V10) and chiller (Lab Tech H50-500)[7].

2.4. Preliminary phytochemical tests

The preliminary phytochemical evaluation was performed for the detection of secondary metabolites by standard procedures[8]. The *A. mexicana* extract was screened for the confirmation of alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, phenols, saponins, and tannins.

2.5. Determination of total phenolic and flavonoids

Total phenolic and total flavonoid contents in *A. mexicana* crude extract were determined according to the standard methods with some modifications[9]. Total phenolic content was determined by the Folin-Ciocalteu method. The solution of 1 mg/mL plant extract was mixed with 2.5 mL of 10% w/v Folin-Ciocalteu reagent. The mixture was quickly agitated for 5 min, followed by subsequent addition of 2 mL of 7% w/v Na_2CO_3. The same procedure was repeated for preparation of the calibration curve with different concentrations of gallic acid. The solutions were incubated for 90 min at 25 ℃ in darkness followed by measuring the absorbance at 750 nm by UV-Vis spectrophotometer (UV-2300, Shimadzu, Tokyo, Japan). The results were expressed as mg of gallic acid equivalents (GAE)/g of *A. mexicana* extract.

Aluminium chloride ($AlCl_3$) calorimetric method was adopted for the estimation of total flavonoids contents. The stock solution (1 mg/mL) of plant extract was made in methanol. Then, 200 μL stock solution, 100 μL of 10% w/v $AlCl_3$, 100 μL of 1 M potassium acetate, and 4.6 mL of distilled water were mixed. The same procedure was performed for preparing the standard curve using different concentrations of quercetin. The blank sample was made by substituting $AlCl_3$ solution with distilled water. All solutions

were filtered, incubated for 45 min at 25 ℃ and absorbance was measured at 415 nm using UV-Vis spectrophotometer. Flavonoid content was calculated from quercetin calibration curve and results were expressed as mg of quercetin equivalent (QE)/g of *A. mexicana* extract.

2.6. Ex vivo experiments

2.6.1. Preparation of isolated rabbit jejunum segment

The abdominal cavity of rabbit (preanesthetized with cholorform) was cut open to remove the intestine. The jejunum was identified, and 2 cm jejunum segment was isolated after removing the mesenteric attachment. The piece of jejunum segment was loaded in the tissue bath filled with 10 mL of Tyrode's solution (pH: 7.4). The tissue bath was bubbled with a mixture of oxygen and carbon dioxide (95%: 5%) at 37 ℃ by applying a stretch tension of 1 g. The periodic rhythmic contractile activity was recorded by an isotonic transducer attached to a power lab data Acquisition system (AD Instrument, Sydney, Australia) by using lab chart version-8[10].

2.6.2. Spasmolytic effect on jejunum segment

The rabbit jejunum segment was stabilized for 30 min with repeated exposure to carbamylcholine (0.3 μM) at 5 min intervals in the tissue organ bath till two identical consecutive contractile peaks were recorded. The plant extract was then added to the issue bath in a cumulative manner (0.001-3.0 mg/mL) to assess its effect on spontaneous contractility of jejunum segments. The plant extract exhibiting contractile activity was further investigated in the presence of muscarinic antagonist (atropine; 0.3 μM). The contractile and relaxant effect of *A. mexicana* was presented in terms of percentage variations in periodic rhythmic contraction of jejunum segment with and without 0.3 μM atropine. The calcium channel blocker verapamil was used as a standard at 0.01-1.0 μM. To investigate the underlying mechanism of the spasmolytic activity, the plant extract was added to jejunum segments pre-contracted with potassium (80 mM). The relaxant responses of *A. mexicana* extract and verapamil were calculated as a percent of initial contractility prior to the addition of 1st dose of *A. mexicana* (0.01 mg/mL) and verapamil (0.001 μM). The potassium (80 mM) opened up the voltage gated *L*-type calcium channels in the jejunum segments. The influx of extracellular calcium (from tyroid's solution) induced the contractions in the jejunum segments[11].

For the evaluation of calcium channel blocking action, potassium (80 mM) was used to depolarize the jejunum preparation by adding to the tissue bath that induced sustained contractions in the smooth muscle of the isolated jejunum segment by the opening of voltage-dependent *L*-type calcium channel. The influx of extracellular calcium produced contractions, whereas the chemical inhibiting the potassium (80 mM)-induced contractions was considered as calcium channel blocker.

2.6.3. Calcium channel blocker activity

To substantiate the calcium channel blocker property of *A. mexicana*, the preparation of jejunum segments was permitted to stabilize in normal Tyrode's solution which was further replaced with calcium-free Tyrode's solution containing ethylenediaminetetraacetic acid (0.1 mM) for 20 min to remove calcium. This solution was then replaced with potassium-rich and calcium-free Tyrode's solution. After 30 min incubation, control concentration-response curves of calcium were constructed. When two control curves of calcium were constructed and found to be superimposable, the isolated jejunum tissue was pretreated with *A. mexicana* extract for 45 min. The concentration-response curves for calcium were reconstructed in the presence of test substance in a dose-dependent manner[12].

2.7. In vivo experiments

2.7.1. Gastrointestinal transit ratio

The charcoal meal experiment was performed according to the method described by Hamid and Janbaz with some modifications[13]. Five groups (5 mice/group) were used. The mice were fasted overnight. The control group was orally administered with normal saline. The standard group was treated with carbamylcholine (1 mg/kg, *i.p.*). The treatment groups received *A. mexicana* extract at 50, 100 and 150 mg/kg *via* gastric gavage needle. After half an hour, each mouse was orally administered with 0.2 mL of charcoal meal (a mixture of 5% deactivated charcoal and 0.5% sodium carboxymethyl cellulose). Half an hour later, all mice were anesthetized with chloroform and killed by cervical dislocation. The abdomens were opened, and the distance travelled by a charcoal meal from the pylorus to the caecum was measured by a scale in centimetres (cm).

2.7.2. Laxative activity

The mice were separated into five groups (5 mice/group) and each mouse was placed in an individual cage. The control group was given saline (10 mL/kg, orally) and the standard group was treated with carbamylcholine (1 mg/kg, *i.p.*). The treatment groups were treated with different doses of *A. mexicana* crude extract (50, 100 and 150 mg/kg, respectively) by an oral route. After 6 h, the cages were inspected to count the total number of wet faecal droppings for each mouse in all groups. Increases in wet droppings comparative to total droppings were recorded and considered as laxative effect[14].

2.7.3. Antiemetic activity

The *A. mexicana* extract was screened for antiemetic effect by copper sulphate ($CuSO_4$)-stimulated emesis in chick model as reported previously[15]. The chicks were arranged in five groups (5 chicks/group) and individually accommodated for acclimatization.

The control group was administered with normal saline (10 mL/kg, orally) and the standard group was treated with chlorpromazine (150 mg/kg, orally). The treatment groups received *A. mexicana* extract orally at doses of 50, 100 and 150 mg/kg, respectively. After 15 min, $CuSO_4$ solution at 50 mg/kg was orally given to all pre-treated chicks. The number of retches was counted in all the groups after 10 min. The minimum number of retches counted was considered as safe from the $CuSO_4$ stimulated emesis.

2.8. Acute oral toxicity test

The acute oral toxicity study was performed in BALB-c albino mice. The mice were randomly arranged into four groups (10 mice in each group). The negative control group was orally given 10 mL/kg saline. Mice in treatment groups were given *A. mexicana* extract in single oral dose of 500, 1 000 and 2 000 mg/kg, respectively. All mice were observed for signs of toxicity, changes in behaviour or mortality for the initial 6 to 48 h and after 14 d[16].

2.9. Statistical analysis

The median estimated response (EC_{50} values) and 95% confidence interval (*CI*) were computed using the software GraphPad Prism®, version-6 (San Diego, California, USA). Concentration versus response curves (CRCs) was measured by non-linear regression sigmoidal response curve with variable slopes. One-way analysis of variance (ANOVA) was applied followed by Dunnett's test for *in vivo* experiments. $P<0.05$ was considered statistically significant.

2.10. Ethical statement

All experiments were approved by the Ethical Committee of the University of Lahore, Lahore (No.EC/Reg No/PHMG 02163005).

3. Results

3.1. Preliminary phytochemical screening

The secondary metabolites including alkaloids, anthraquinones, flavonoids, glycosides, phenolic, saponins, and tannins were detected in *A. mexicana* extract.

3.2. Total phenolic and flavonoid contents

The phenolic content of *A. mexicana* crude extract, measured from standard calibration curve of gallic acid ($y = 0.005\,1x + 0.048$, $r^2 = 0.993\,5$) by Folin–Ciocalteu method was (267.75 ± 5.77) mg GAE/g, whereas, the total flavonoid content determined from quercetin calibration curve ($y = 0.003\,3x - 0.011$, $r^2 = 0.991\,9$) was estimated as (73.86 ± 6.01) mg QE/g.

3.3. Ex vivo experiments

3.3.1. Spasmogenic effect on isolated jejunum fragment

The *A. mexicana* extract enhanced periodic contractile activity in jejunum segments of rabbits in the tissue bath at 0.01-0.3 mg/mL with an EC_{50} value of 0.016 mg/mL (95% *CI*: 0.009-0.029 mg/mL). At 1 mg/mL concentration of *A. mexicana* extract, the periodic contractile response was suppressed in the jejunum segments of rabbit (Figure 1A). The observed contractile effect in the rabbit jejunum segment by *A. mexicana* extract was blocked by atropine (0.3 μM) exposure. In the presence of atropine (0.3 μM), *A. mexicana* crude extract relaxed the rhythmic contraction of the rabbit jejunum segments with an EC_{50} value of 2.18 mg/mL (95% *CI*: 1.29-3.68 mg/mL). The standard drug (verapamil) suppressed the periodic contraction in rabbit jejunum segments at 0.01-1 μM with an EC_{50} value of 0.46 μM (95% *CI*: 0.25-0.85 μM) (Figure 1 B-D).

3.3.2. Spasmolytic effect on potassium induced contractions

The *A. mexicana* crude extract relaxed the potassium (80 mM)-induced contractility in rabbit jejunum segments with an EC_{50} value of 9.07 mg/mL (95% *CI*: 5.74-14.34 mg/mL). In contrast, the standard drug (verapamil) showed the remarkable spasmolytic effect with an EC_{50} of 1.80 μM (95% *CI*: 1.05-3.08 μM) (Figure 2).

3.3.3. Calcium channel blocker activity

For confirmation of calcium channel blocking activity of *A. mexicana* crude extract, calcium concentration-response curves were constructed in the absence and presence of the extract. *A. mexicana* crude extract (1.0-5.0 mg/mL) and verapamil (0.1-1.0 μM) shifted the concentration-response curves of calcium in rabbit jejunum segments to the right in a non-parallel manner (Figure 3).

3.4. In vivo experiments

3.4.1. Effect of A. mexicana extract on gastrointestinal transit ratio

The gastrointestinal transit ratio of a charcoal meal model in mice was used for determination of peristaltic action. The charcoal meal distance propelled by the control group (NaCl; 0.9%) was (18.52 ± 0.28) cm. Similarly, pre-treatment of mice (standard group) with carbamylcholine (1 mg/kg) pushed the charcoal meal from the pylorus of stomach to a distance of (30.38 ± 0.77) cm in the intestine. The distance of charcoal meal movement was gradually increased with the increasing concentrations of the extract in the treatment groups. The results indicated that *A. mexicana* crude extract exhibited dose-dependent prokinetic activity ($P < 0.05$) in contrast to the negative control and was comparable to the carbamylcholine group (Figure 4).

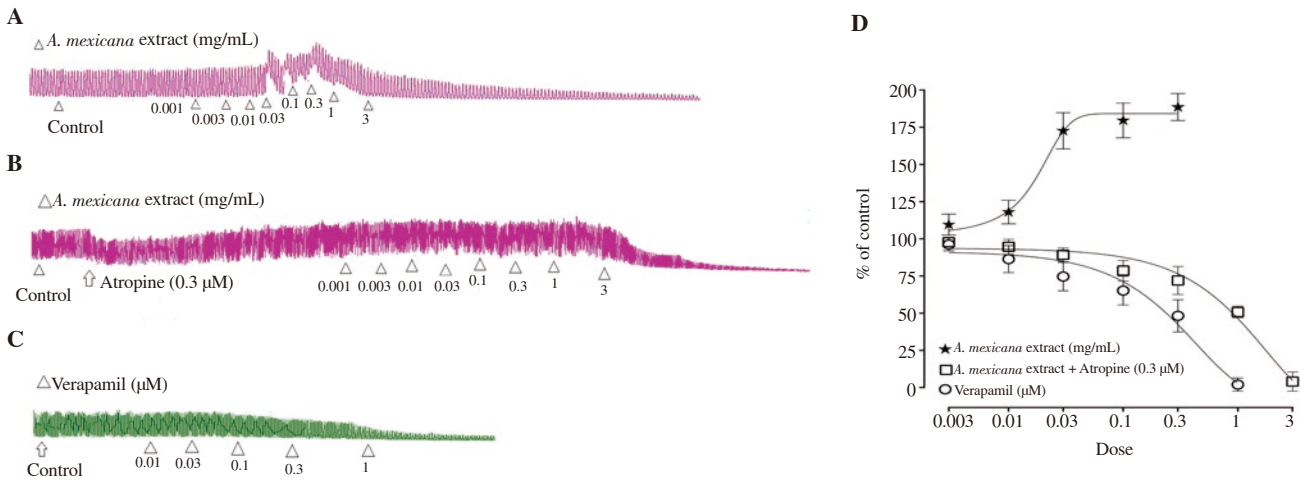

Figure 1. Spasmogenic and spasmolytic effect of *Argemone mexicana* (*A. mexicana*) crude extract on rhythmic contraction of rabbit jejunum fragment. (A) Spasmogenic effect of *A. mexicana* crude extract at tissue bath concentrations of 0.001-0.3 mg/mL. (B) Blockage of spasmogenic effect of *A. mexicana* crude extract with atropine (0.3 μM). (C) Spasmolytic effect of standard calcium channel blocker (verapamil). (D) The spasmogenic or spasmolytic effect of the extract in the absence and presence of atropine. Data are presented as mean ± SD (n=5).

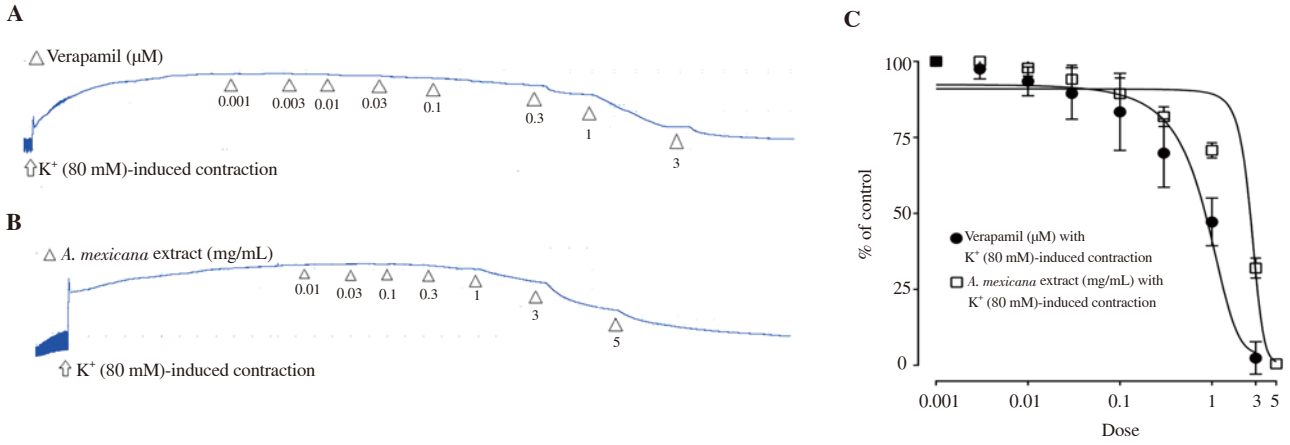

Figure 2. Spasmolytic effect of (A) verapamil and (B) *A. mexicana* crude extract on K^+ (80 mM)-induced contraction in isolated rabbit jejunum fragment. (C) The relaxant effect of *A. mexicana* crude extract and verapamil on K^+ (80 mM)-induced contraction as percentage of control. Data are expressed as mean ± SD (n=5).

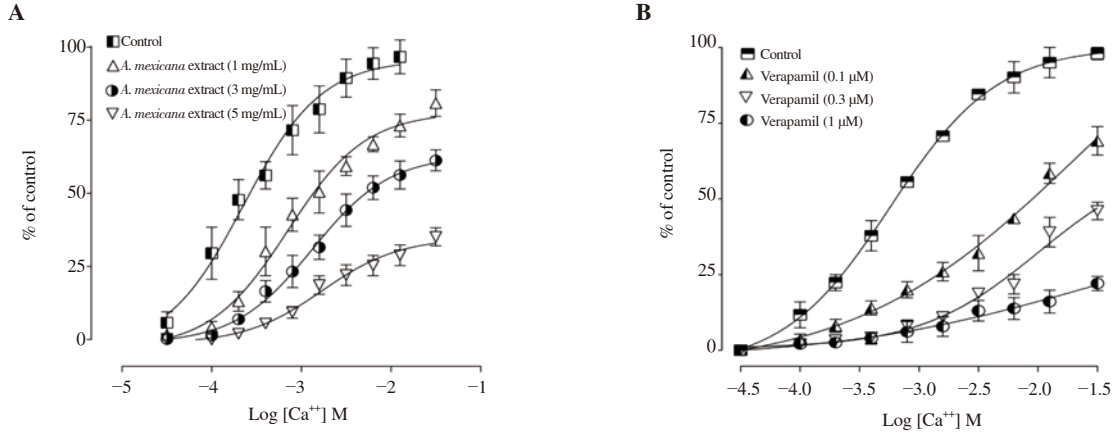

Figure 3. Concentration response curves of Ca^{++} in the presence of (A) *A. mexicana* crude extract (1-5 mg/mL) or (B) verapamil (0.1-1.0 μM). Data are expressed as mean ± SD (n=5).

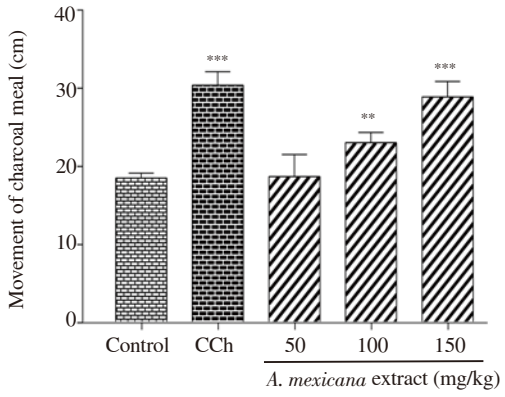

Figure 4. The effect of *A. mexicana* crude extract and carbamylcholine (CCh; 1 mg/kg) on transit distance of charcoal in the gastrointestinal tract of mice in charcoal meal experiments. Data are expressed as mean ± SD and analyzed by one-way ANOVA (Dunnett's test). $^{**}P<0.01$ and $^{***}P<0.001$ represent significant difference compared with the control group.

3.4.2. Laxative activity of A. mexicana extract

The mice administered with *A. mexicana* extract at doses of 50, 100 and 150 mg/kg showed laxative effect by increasing the number of wet faecal droppings in comparison to that of the control group. Similarly, carbamylcholine (1 mg/kg) increased the number of wet faecal droppings (Figure 5).

3.4.3. Antiemetic activity of A. mexicana extract in chicks

The number of retches in chicks (standard group) treated with oral solution of chlorpromazine (150 mg/kg) was significantly decreased as compared with the control group. Similarly, *A. mexicana* extract reduced the number of vomiting in a dose-dependent manner, which was comparable to the standard group (Figure 6).

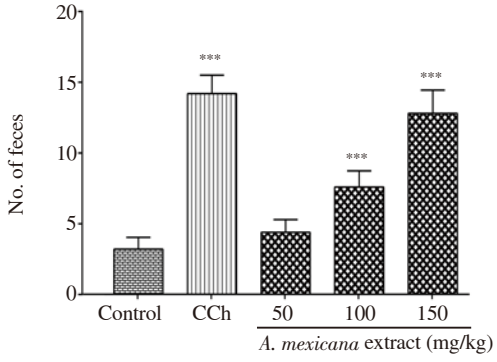

Figure 5. The effect of *A. mexicana* crude extract and carbamylcholine on the number of wet faecal dropping in mice. Data are expressed as mean ± SD and analysed by one-way ANOVA (Dunnett's test). $^{***}P<0.001$ represents significant difference compared with the control group.

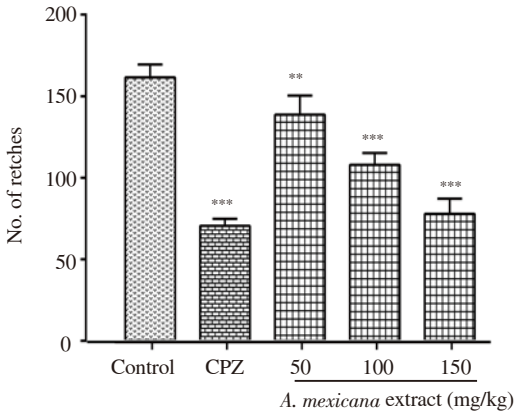

Figure 6. The effect of *A. mexicana* crude extract and chlorpromazine (CPZ; 150 mg/kg) on $CuSO_4$ (50 mg/kg) stimulated retches in chicks. Data are expressed as mean ± SD and assessed by one-way ANOVA (Dunnett's test). $^{**}P<0.01$ and $^{***}P<0.001$ represent significant difference compared with the control group.

3.5. Acute oral toxicity study

The mice treated with *A. mexicana* extract at 500 and 1 000 mg/kg exhibited no sign of toxicity and mortality until 14th day. However, treatment with *A. mexicana* extract at 2 000 mg/kg displayed minor signs of toxicity including reduction in locomotion, ruffled skin and increased number of wet faeces which disappeared after 48 h.

4. Discussion

A. mexicana Linn is used as a traditional remedy for gastrointestinal problems including indigestion, constipation, nausea, and vomiting with carminative, spasmogenic, laxative and antiemetic activities[17]. Therefore, the present study was undertaken to validate the laxative and antiemetic activities of the *A. mexicana* crude extract.

The *A. mexicana* extract gradually increased the periodic contractile activity of isolated rabbit jejunum fragment at 0.01-0.3 mg/mL concentration but decreased the activity at concentration from 1.0 mg/mL to 3.0 mg/mL, indicating its spasmogenic activity. The underlying mechanism can be explained by the physiological function of the gastrointestinal tract. Motility is the basic physiological function of the gastrointestinal tract. Contractile activities of gastric smooth muscles varied in different regions of the gastrointestinal tract with oscillating depolarization (slow-wave) and rapid depolarization (spike). Muscles of the gastrointestinal tract are enriched with interstitial Cajal cells linked to the smooth muscle and enteric nerves. The Cajal cells act as a pacemaker to generate an action potential in the smooth muscles[18].

Acetylcholine is an excitatory neurotransmitter of enteric nerves. Physiologically, the role of acetylcholine in the gastrointestinal tract is to maintain the peristaltic rhythm by activating the multiple types of muscarinic receptors especially sub-type of muscarinic receptor

M_1 and M_3. These receptors are metabotropic and belong to a G-protein coupled receptors linked with an enzyme phospholipase-C, which on activation induces the secondary messengers such as 1,3,5-inositol triphosphate (IP_3) and diacylglycerol. The IP_3 releases the stored calcium from the sarcoplasm of the intestinal cells, whereas diacylglycerol mediates the phosphorylation of cell proteins. At the same time, voltage-dependent *L*-type Ca^{++} channels located in cell membrane on intestinal smooth muscles were activated and Ca^{++} influx was increased from the extracellular spaces. The increased intracellular Ca^{++} level in smooth muscles induced a strong contraction in the gastrointestinal tract resulting in the propulsion of the intestinal contents[19]. The plant extract exhibited acetylcholine like activity to stimulate the intestinal muscarinic receptor and produced the spasmogenic effect. The presence of acetylcholine like property in the extract was confirmed by blockade of spasmogenic effect by atropine (0.3 μM) and the extract produced the spasmolytic effect in jejunum fragments. The spasmolytic effect of the extract indicated that it contained some constituents that had calcium channel blocking activity. This was confirmed by the relaxant effect of the extract on potassium (80 mM)-induced contraction in jejunum fragments. The extracellular potassium at higher concentration depolarized the jejunum fragment by opening the voltage-gated *L*-type calcium channels and induced the sustained contractions in the smooth muscles of jejunum fragment by the influx of extracellular calcium. The plant extract relaxed the potassium (80 mM)-induced contraction like verapamil (a standard calcium channel blocker), clearly indicating that it blocked the *L*-type calcium channels and prevented the calcium influx. The calcium channel blocking activity of the extract was further confirmed by the rightward shift of the concentration-response curve of calcium similar to verapamil[20].

The spasmogenic activity of the plant extract was further proved by *in vivo* animal testing. The plant extract was tested for its possible gastrointestinal stimulant (spasmogenic) effect in the small intestine of mice by charcoal meal movement and production of wet faecal mass in comparison to saline-treated mice. The plant extract exhibited significant dose-dependent intestinal stimulation and production of the wet faecal mass that was similar to the carbamylcholine. Carbamylcholine is used as a standard cholinergic agonist and gastrointestinal stimulant agent in *in vivo* mice model. Previously, it was reported that the gastrointestinal laxative effect of herbal remedies was usually mediated *via* acetylcholine like prokinetic mechanism and *A. mexicana* is one of them[21].

The plant was initially screened for the preliminary phytochemicals that confirmed the existence of active constituents such as alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins, and tannins. The phenols and flavonoids are the unique plant phytochemicals that have well established antioxidant, anticancer, anti-inflammatory, cardioprotective, bronchodilator, and gut modulatory properties[22,23]. Flavonoids were reported to be useful in gastrointestinal problems *i.e.*, constipation and act *via* anion secretion across the gastrointestinal tract by influencing its mucosa. Some citrus fruit-derived flavonoids like naringenin were presumed to be potent stimulators of cAMP-dependent chloride secretion in colonic epithelium and produce laxative effect with significant improvement of constipation[24]. The plant extract also contained anthraquinones which locally acted on the epithelial cells of the large intestine causing alterations in absorption as well as excretion of fluid and electrolytes, in addition to the enhanced motility of the gut, leading to the laxative effects. The mechanism of action of anthraquinones was proposed to inhibit the colonic sodium/potassium-adenosine triphosphatase, resulting in sodium and water retention and increasing the paracellular permeability of the colonic mucosa[25].

Emesis is an unpleasant response associated with motor activity of the gastrointestinal tract resulted in emitting of gastrointestinal content through mouth. The *A. mexicana* extract exhibited antiemetic effect in a dose-dependent manner through prevention of $CuSO_4$-stimulated emesis in chicks, similar to the chlorpromazine. The antiemetic activity of the plant extract might be attributed to its phytochemical constituents which had blocked the dopamine receptors in chemoreceptor trigger zone as well as in the peripheral gastrointestinal tract[26]. Moreover, the acute oral toxicity study was performed in mice at the maximum dose of 2000 mg/kg. The plant extract was found to be safe without any mortality and clinical sign of toxicity till the end of study period.

In conclusion, the preliminary phytochemical analysis of *A. mexicana* Linn confirmed the presence of alkaloids, anthraquinones, flavonids and phenols. These phytochemicals were responsible for the laxative and antiemetic effect of the plant extract through possible mechanisms of cholinergic agonist and calcium channel blocker activities which justifies its use for the treatment of gastrointestinal problems including constipation and emesis. The current study presented a rationale for the safe and effective medicinal use of *A. mexicana* in the treatment of constipation and emesis, however, further studies such as metabolomics studies of the plant extract and bioactivity based isolation may be carried out to verify the ethnomedical uses of the plant extract.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Authors' contributions

RI, IH, KHJ and MFA conceptualized the study and acquired the data. MFA, A Saleem, A Sharif, SP, BA and SA designed the study. KHJ, MFA, A Saleem and SP analyzed the data. RI, IH, MFA, A Saleem, A Sharif, KS, SP, BA and SA carried out the experimental work. RI, IH, MFA and A Saleem wrote the manuscript. KHJ, SP, SA, KS, BA and A Sharif critically reviewed the manuscript.

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