Antispasmodic and antidiarrhoeal activity of the fruit of Rosa moschata (J)

Niaz Ali1*, Hina Alam1, Aslam Khan1, Ghayour Ahmed2, Wadood Ali Shah3, Muhammad Nabi1 and Muhammad Junaid3

Abstract

Background: The fruit of Rosa moschata has traditionally been used for the treatment of abdominal spasm and diarrhoea. Therefore, the aim of this study was to investigate mechanism(s) responsible for its medicinal use in gut spasm and diarrhea.

Methods: Hydro-methanolic extract of Rosa moschata (Rm.Cr) was studied in isolated rabbit’s jejunal preparations for possible antispasmodic activity. Based upon in vitro relaxant activity in isolated gut preparations, in vivo antidiarrheal activity was carried out in mice to confirm its antidiarrheal effect. Acute toxicity study was performed to determine safe dose range before in vivo experiments.

Results: In isolated rabbits’ jejunal preparations, Rm.Cr inhibited the spontaneous and high K+-induced contractions with respective EC50 values of 0.66 (0.44-0.97; n = 5) and 2.28 mg/mL (1.43-3.62; n = 5), like that of verapamil. This suggests the presence of calcium channel blocking (CCB) activity as a possible mode of action. The Ca++ channel blocking activity was further confirmed when pre-treatment of isolated jejunums with Rm.Cr (1-5 mg/mL) caused a rightward shift in the Ca++ concentration-response curves (CRCs), similar to verapamil. Rm.Cr was safe up to 2000 mg/kg for in vivo acute toxicity. Rm.Cr provided 55% and 80% protection from diarrhoea in respective doses of 100 mg/kg and 1000 mg/kg. These data indicates that the crude extract of Rosa moschata possesses Ca++ antagonist-like constituent(s), which explains its inhibitory effect on gut motility; a mechanism that underlies its antidiarrheal and antispasmodic activities.

Conclusion: The study shows that the crude extract of fruits of Rosa moschata possesses antispasmodic effects mediated possibly through voltage gated Ca++ channel blockade, which provides sound pharmacological base to its medicinal use in gut spasms and diarrhoea, though additional mechanism(s) cannot be ruled out.

Keywords: Rosa moschata, Antidiarrhoeal, Antispasmodic, Ca++ antagonist, Verapamil

Background

Plants have been used from a long while for the treatment of various diseases and 80,000 species out of 250,000 of higher plants are used medicinally [1]. The use of natural products are considered safe as compared to synthetic products, so the beliefs on using the synthetic products is decreasing compared to natural products [2]. Herbal medicines show potential uses in future because most of the plants, their activities and pharmacological activities have not being explored completely [3].

* Correspondence: niazpharmacist@yahoo.com
1Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan
Full list of author information is available at the end of the article

© 2014 Ali et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
antioxidant property has been explored on scientific background [15].

The fatty acid composition of *Rosa moschata* has been determined by gas chromatography and then confirmed by the gas chromatography mass spectroscopy (GC-MS) which contains stearic acid, palmitic acid, oleic acid, margaric acid, linoleic acid and linolenic acid. The other isolated compounds of *Rosa moschata* are Vitamin A, C, E, flavonoids and essential oil [5,16,17]. Other species of the genus rosa like *Rosa damascena* have been reported to have spasmodenic (low concentration) and spasmolytic activity (in higher concentration) on rats’ ileum [18]. In addition, relaxant activity of *Rosa damascena* on guinea pig tracheal chains has been reported [19].

Due to its medicinal use in gut spasm and diarrhea, the current work focused to provide pharmacological basis for the medicinal use of *Rosa moschata* in gut spasms.

**Methods**

**Plant materials and extraction of crude extract**

Fresh fruits of *Rosa moschata* were collected from Malakand region and authenticated by Professor Dr. Jehandar Shah, ex vice chancellor and plant taxonomist, University of Malakand, Pakistan. A voucher specimen (RM-2103) has been deposited in the Department of Pharmacology, Khyber Medical University, Peshawar.

Plant materials, free of adulterants, were repeatedly extracted (3 times) with commercial grade methanol (80%) at room temperature and the combined extract was evaporated in rotary evaporator at 35-40°C to a semisolid mass, the crude extract of *Rosa moschata*. The extract (free of solvent) was solubilized in normal saline and distilled water for the *in-vivo* and *in-vitro* experiments, respectively.

**Drugs and standards**

Analytical grade chemicals were used throughout these experiments. Acetylcholine was purchased from BDH, Poole, England, which was used for the maintenance of tissues at quiescent doses. Rest of the chemicals were of E Merck grade. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh in normal saline on the days of experiments.

**Animals**

BALB/C mice (weighing 25-30 g, hired from NIH, Islamabad) and local breed rabbits (weighing 1.5-2 kg) of either sex were housed at the animal house of the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan under a controlled environment (23-25°C). The animals were kept in respective cages and were fasted overnight before starting the experiments. Advanced Study & Research Board and Ethical Board of Khyber Medical University approved the study protocols (ASRB000152/AA/IBMS/20/03/2014).

**Preliminary phytochemical screenings**

Plant extracts was tested for various active principles i.e. Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, and Carbohydrate using different tests such as Liebermann Burchard test was used for steroids and triterpenoids, Keller Killiani and Bromine water test for Glycosides, Foam test for Saponins, Hager’s test for Alkaloids, Ferric chloride test, Alkaline reagent test and Lead acetate solution test for Flavonoids, Gelatin test for Tannins, Biuret test for proteins and Benedict’s test for carbohydrates described by Bhaddray, 2012 [20].

**Acute toxicity**

Acute toxicity was performed as reported previously by Ali et al. 2013 [21]. Briefly, animals were divided in groups of 5 mice in each group. The test was performed in test doses of 1, 10, 1000 and 2000 mg/kg, given through intraperitoneal route. Another group of mice was administered normal saline which served as negative control. The mice were allowed food and water ad libitum during 24 hours test period. The animals were under regular observations for gross behavioral changes and mortality during the said period.

**Isolated tissue preparations**

The isolated tissues experiments were performed in accordance with protocols established in our lab [21,22]. Rabbits were fasted for 24 hours before the experiments with free access to water. Then rabbits were sacrificed by cervical dislocation, the abdomens were cut open and the jejunal portions were isolated. Preparations of about 2 cm long were mounted in 15 mL tissue baths containing Tyrode’s solution maintained at 37°C, constantly aerated with carbogen (a mixture of 5% carbon dioxide in oxygen). The composition of Tyrode’s, in mM, was: NaCl 136.9, KCl 2.7, NaHCO3 11.9, MgCl2 1.1, Glucose 5.6, NaH2PO4 0.4, and CaCl2 1.8 (pH 7.4). Tension of about 1.0 g of preload was applied to tissues and subsequently kept undisturbed for 30 min for stabilization. Upon stabilization of the isolated jejunal tissues, reproducible control responses of acetylcholine (0.3 μM) were obtained.

Under these experimental conditions, rabbits’ jejunos exhibited spontaneous contractions, allowing testing for possible relaxant (spasmolytic) activity directly without use of a spasmogen or an agonist.

**Calcium antagonist activity**

To assess whether the spasmolytic activity of the test substances was mediated through voltage gated Ca++ channels blockade, high concentration of K+ (80 mM), as KCl, was used to depolarize the preparations [21,22], which
produced sustained contractions. Cumulative dosing of verapamil (standard) and extract of Rm.Cr was then added to isolated tissue baths to obtain concentration-dependent inhibitory responses. The relaxation of isolated jejunal tissues, pre-contracted with K+ was expressed as percent of the control pre-contractions.

Ca++ channel blocking activity of plant extract was confirmed when the tissues were first stabilized in normal Tyrode’s solution and normal Tyrode’s solution was replaced with Ca++ free Tyrode’s solution containing EDTA (0.1 mM) for 30 minutes. EDTA chelates the Ca++. This solution was then replaced with K+-rich and Ca++-free Tyrode’s solution, having the following composition: NaCl 91.04, KCl 50, NaHCO3 11.90, MgCl2 1.05, glucose 5.55, NaH2PO4 0.42 and EDTA 0.1 mM. Control concentration-response curves (CRCs) of Ca++ were obtained after an incubation period of 30 minutes. When the CRCs of Ca++ were found superimposable (usually after two cycles), the tissues were then pretreated with Rm.Cr for 60 minutes to test the possible Ca++ channel blocking effect. The CRCs of Ca++ were reconstructed in the presence of different concentrations of the plant extract and verapamil (standard).

**Data recording and Interpretation**

Isotonic Transducer (MLT 0210/A Pan Lab) were used to record the intestinal recordings, connected with Power lab (Model No: 4/26 T) AD Instruments, Australia. Bridge Pod Amplifier connected with the Power lab was used for amplification of the intestinal responses.

Lab Chart 7 supplied with the power Lab was used to record and interpret the data.

**Effects on castor oil-induced diarrhea**

Based on positive relaxant activity of Rm.Cr on gut motility, the in-vivo anti-diarrheal activity of the extract was carried out following the methods as described in our previous articles with slight modifications [23]. In this study, Balb C albino mice were kept on overnight fasting. Five groups of the mice were made and kept in five different steel cages containing four mice in each. Bottom of each cage was covered with blotting sheet. Normal saline (10 mL/kg, orally) was given to first group and labeled as the negative control. Rm.Cr was administered orally by intra-gastric feeding needle to the second, third and fourth group in doses of 100, 300 and 1000 mg/kg keeping in view the acute toxicity results. Loperamide (10 mg/kg p.o.) was given to the fifth group as positive control. 10 mL/kg of castor oil (p.o.) was given to all the groups after one hour post extract/test sample treatment. All the groups were then observed for the presence of diarrhoeal droppings in the individual cages for 4 hours after ingestion of the castor oil. Based on the number of dry and wet feces, percent protection against the castor oil-induced diarrhoea was calculated for each group.

**Data analysis**

Data are expressed as mean ± standard error of the mean (SEM) and the median effective concentrations (EC50) values were given with 95% confidence intervals (CI) using Graph Pad Prism.

| s. no | Phytochemicals | Remarks |
|-------|----------------|---------|
| 1.    | Alkaloids      | -       |
| 2.    | Flavonoids     | +       |
| 3.    | Tannins        | +       |
| 4.    | Saponins       | +       |
| 5.    | Carbohydrate   | +       |
| 6.    | Quinones       | -       |
| 7.    | Sterols        | -       |
| 8.    | Phenolics      | +       |
| 9.    | Proteins       | +       |
| 10.   | Terpenoids     | +       |
| 11.   | Glycosides     | -       |

**Table 1 Phytochemical screenings of Rosa moschata**

![Figure 1](http://www.biomedcentral.com/1472-6882/14/485)
Results and discussion

Upon preliminary phytochemical screenings, *Rosa moschata* tested positive for presence of tannins, flavonoids, saponins, phenolics, proteins and terpenoids. It tested negative for alkaloids, quinones, sterols and glycosides (Table 1). Acute toxicity results proved that the extract is safe up to 2000 mg/kg. The percent survivors are expressed in Figure 1. This reflects good safety profile of the sample making it a possible candidate for the development of herbal products.

As there is no study available on the antispasmodic and anti diarrheal activity of this plant, therefore, this study was undertaken to provide the pharmacological basis for its medicinal use in hyperactive gut disorders and to investigate the mechanism of its spasmolytic effect. For this purpose this study was carried out on gut motility in isolated spontaneously contracting rabbits' jejunums, where cumulative addition of crude extract of *Rosa moschata* caused concentration-dependent inhibition of the spontaneous and high K⁺ induced contractions, with EC₅₀ values of 8.21 mg/mL (6.66 - 10.12) and 3.93 (3.39 - 4.55), respectively (Figure 2A). In similar pattern, verapamil, a standard Ca⁺⁺ antagonist [22], relaxed the spontaneous and high K⁺ induced contractions, with EC₅₀ values 0.51 mg/mL (0.43 – 0.60) and 0.16 mg/ml

![Figure 2](http://www.biomedcentral.com/1472-6882/14/485)

![Figure 3](http://www.biomedcentral.com/1472-6882/14/485)
This shows smooth muscle relaxant (antispasmodic) activity may be mediated through calcium antagonistic effect as high K⁺ (>30 mM) is known to cause smooth muscle contractions through opening of voltage-dependent L-type Ca⁺⁺ channels, thus allowing influx of extracellular Ca⁺⁺ causing a contractile effect [24] and the substance which cause inhibition of high K⁺ -induced contraction is considered to be an inhibitor of Ca⁺⁺ influx [25]. Extract of the *Rosa maschata* relaxed the high K⁺ -induced contractions, similar to that caused by verapamil (standard Ca⁺⁺ antagonist [26] indicating its CCB action. The Ca⁺⁺ antagonist effect of *Rosa moschata* was further confirmed when Rm.Cr dose dependently (1–5 mg/mL) shifted the Ca⁺⁺ concentration response curves to the right (Figure 3A), like that caused by verapamil (Figure 3B). Ca⁺⁺ antagonists have been shown to be beneficial in gut disorders resulting from hyperactivity such as diarrhea and abdominal cramps [27]; hence the observed CCB effect justifies the medicinal use of *Rosa moschata* in such conditions. This observed Calcium channel blocking effect of the plant may be due to the presence of flavonoids, as evident from phytochemical screening, because the constituents of this class of compounds have been reported to have Calcium channel blocking activity [28,29], however, contribution of other compound may not be ruled out. This is the first functional study on the gut motility with possible mode of action, carried out on the fruit of *Rosa moschata*.

Based on the relaxant effect of *Rosa moschata* on spontaneous and K⁺ induced contractions in isolated jejunal preparations, an in vivo model was used to test the extract for possible inhibitory effect on gut motility as for possible antidiarrhoeal activity. The crude extract provided protection from diarrhea in castor oil-induced diarrhea, similar to loperamide, a standard antidiarrheal agent [30]. Both extract and loperamide significantly inhibited (P < 0.05) the frequency of defection as well as wetting of feces when compared with the untreated group (i.e. mice which only received castor oil but neither crude extract, nor loperamide). The percent protection provided by the crude extract was 25 ± 5, 55 ± 5 and 80 ± 13.2 at doses of 30, 100 and 1000 mg/kg respectively. Loperamide provided 100% protection at 10 mg/kg (Figure 4).

Hydrolysis of castor oil results in the formation of ricinoleic acid [31], which produces changes in the transport of water and electrolytes resulting in a hyper secretory response and generation of a giant contraction of the intestine [32]. Thus, a potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting either contraction of smooth muscles of the gut or electrolyte out flux [33].

**Conclusion**

In summary, this study shows that the crude extract of *Rosa moschata* possesses antispasmodic and antidiarrheal effects, mediated possibly through Ca⁺⁺ channel blockade, which provides sound pharmacological base to its medicinal use in diarrhoea and gut spasms, though additional mechanism(s) cannot be ruled out.

**Abbreviations**

Rm.Cr: Crude methanolic extract of *Rosa moschata*; CCB: Calcium channel blocking; CRCs: Concentration-response curves.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

NA: Data interpretation, preparation of the manuscript. HA: Performed antispasmodic activity (M.Phil Scholar). AK: helped in constructing calcium chloride curves and manuscript preparation. GA: Data acquisition. SWAS: Data acquisition and helped in Phytochemistry determination. MN: Data acquisition of antidiarrhoeal activity. MJ: helped in Phytochemistry. All the authors have read and approved the final proof of the manuscript.

**Acknowledgements**

The author thanks Professor Dr. Jehandar Shah for identifying the plant. The authors want to thank the Higher Education Commission of Pakistan for providing research grant no: 20-1723/R&D/10 for establishment of pharmacology laboratory at IBMS, KMU.

**Author details**

1Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan. 2Department of Pharmacy, Kohat University of Science and Technology, Kohat, Pakistan. 3Department of Pharmacy, University of Malakand, Chakdara Dir, Lower, Pakistan.

Received: 9 September 2014 Accepted: 10 December 2014 Published: 13 December 2014
References

1. Fabricant DS, Farnsworth NR: The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001, 109:69.
2. Jay P, Thomas J, Mathew S, Skaria BP: Medicinal plants. Trig Horticulture 1998, 2:49–62.
3. Hassan BAR: Medicinal Plants (Importance and Uses). Pharma Acta Acad 2012, 3:10.
4. Barkatullah B, Ibrar M: In vivo and phytoeconomic elaboration of Lilownai valley, district Shangla wildlife sanctuary, mussoorie, garhwal himalaya. Himalayan Ecol 2012, 6:1046–9.
5. Sharma P, Devi U: Ethnobotanical uses of Biofencing Plants in Himachal Pradesh, Northwest Himalaya, Pakistan. J Biol Sci 2013, 16:957–63.
6. Sher H, Elyemeni M, Sher H, Hussain K: Ethnobotanical and economic observations of some plant resources from the Northern Parts of Pakistan. Ethnobotany Res Appl 2011, 9:27–41.
7. Aujal S, Mohammad S, Zahid K, Bakht Z, Habib A, Alam M: Ethnemedicinal and phytoeconomic elaboration of Lilownai valley, district Shangla Pakistan. Int J Res Pharm 2012, 3:164–9.
8. Dutt B, Sharma S, Sharma K, Gupta A, Singh H: Ethnobotanical survey of plants used by Gaddi tribe of Bharmour area in Himalach Pradesh. Himalayan Ecol 2011, 19:22.
9. Kumar A, Mitra M, Singh G, Rawat G: An inventory of the flora of binog wildlife sanctuary, mussoorie, garhwal himalaya. 2012, 21(1):281–299.
10. Afzal N, Rashid A, Munir W, Bergmeier E: Diversity and use of ethnomedicinal plants in the region of Swat, North Pakistan. J Ethnobiol Ethnomed 2013, 9:25.
11. Ali H, Sannai J, Sher H, Rashid A: Ethnobotanical profile of some plant resources in Malam Jabba valley of Swat, Pakistan. J Med Plants Res 2011, 5(6):76–87.
12. Khare CP: Indian Medicinal Plants: An Illustrated Dictionary. Springer. 2007.
13. Jehanzeb K, Rooman K, Rizwana AQ: Ethnobotanical study of commonly used weeds of District Bannu, Khyber Pakhtunkhwa (Pakistan). J Med Plant Stud 2013, 2:1–6.
14. Franco D, Pinelo M, Sineiro J, Núñez MJ: Calcium antagonism and calcium entry blockade. Pharmacol Rev 1993, 45(1):105–49.
15. Matthews V: New Plantsman: Royal Horticultural Society. 1994.
16. Honavar M, Javidiya K, Khosh-Kheli M: Essential oil composition of fresh and dried flowers of Rosa moschata from Iran. Chem Nat Compd 2011, 47:826–8.
17. Sadraei H, Asghari G, Erami S: Inhibitory effect of Rosa damascena Mill flower essential oil, geraniol and citronellol on rat ileum contraction. Res Pharm Sci 2013, 8(1):17–23.
18. Boxkabady MH, Kiani S, Rakhshandah H: Relaxant effects of Rosa damascena on guinea pig tracheal chains and its possible mechanism(s). J Ethnopharmacol 2006, 106(3):377–82.
19. Bhandary SK, Kurni N, Bhat VS, Shamila K, Bekal MP: Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. J Health Sci 2012, 2:35–8.
20. Ali N, Shah I, Shah SW, Ahmed G, Shoaib M, Junaid M, Ali W, Ahmed Z: Antioxidant and relaxant activity of fractions of crude methanol extract and essential oil of Artemisia macracantha jacquem. BMC Complement Altern Med 2013, 13:96.
21. Ali N, Ahmed G, Shah SW, Shah I, Ghia M, Khan I: Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of Callistemon citrinus curtis. BMC Complement Altern Med 2011, 11:99.
22. Gilani AH, Rehman NU, Mehmood MH, Akhtar KM: Species differences in the antidiarheal and antispasmodic activities of Lepidium sativum and insight into underlying mechanisms. Phytother Res 2013, 27(7):1086–1094.
23. Farre AJ, Colonno M, Fort M, Gutierrez B: Differential effects of various Ca2+ antagonists. Gen Pharmacol 1991, 22(1):77–81.
24. Bolton TB: Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 1979, 59:606–718.
25. Godfraind T, Miller R, Wibo M: Calcium antagonism and calcium entry blockade. Pharmacol Rev 1986, 38(3):411–416.
26. Fleckenstein A: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu Rev Pharmacol Toxicol 1977, 17:149–66.
27. Pasricha PJ: Treatment Of Disorders Of Bowel Motility And Water Flux. In The Pharmacological Basis Of Therapeutics. Edited by Brunton LL, Lazo JS, Parker KL. New York: McGraw-Hill New York; 2006:983–1008.
28. Iwao I, Gould RJ, Snyder SH: Loperamide: blockade of calcium channels as a mechanism for antiarrhythmic effects. J Pharmacol Exp Ther 1984, 231(2):32–32.
29. Croci T, Landi M, Izzo A, Air X, Fur G, Maffiandi JP, Manara L: Role of tachykinins in castor oil diarrhea in rats. Br J Pharmacol 1997, 121:375–80.
30. Pasricha PJ: Treatment Of Disorders Of Bowel Motility And Water Flux. In The Pharmacological Basis Of Therapeutics. Edited by Brunton LL, Lazo JS, Parker KL. New York: McGraw-Hill New York; 2006:983–1008.
31. Carson RL, Young JS, Gershman RA, Jilaihawi O: Calcium channel blockers. Pharmacol Rev 2006, 58(2):91–126.