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Relaxant activity of different fractions of fruits of *Rosa moschata* J.

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Abstract: The current work is an attempt to know that in which fraction(s) the relaxant constituents of *Rosa moschata* concentrate. Crude methanolic extract of *Rosa moschata* was prepared as per our reported procedure. Sub fractions of methanol extract were extracted with different solvents in increasing order of polarity i.e. *n*-hexane > chloroform > ethyl acetate > *n*-butanol > residual aqueous fractions. Different concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5 and 10 mg/ml) of the fractions were tested on spontaneous contractions and KCl induced contractions on rabbits' jejunal preparations. Calcium Concentration Response Curves (CCRCs) in the presence and absence of the test fractions using verapamil were constructed to understand its mechanisms. EtOA fraction was more relaxant with EC_{50} values 0.812±0.149 mg/ml on spontaneous and 2.01±0.08 mg/ml on KCl induced contractions. we also found right shift in its EC_{50} values expressed as log $[Ca^{++}]$M values. In presence of 0.3 mg/ml EtOA fraction, its EC_{50} value was -2.22±0.035 *vs* control EC_{50} -2.71±0.21. For *n*-BuOH fraction, EC_{50} value was -1.82±0.00 *vs* control with EC_{50} -2.28±0.049 at concentration of 0.3 mg/ml. Ethyl acetate fraction of *Rosa moschata* was more potent and is therefore can be a target for activity guided isolation of calcium channel antagonists.

Keywords: *Rosa moschata* J., fractions of *Rosa moschata,* verapamil, calcium channel blocking, relaxant activity.

INTRODUCTION

The importance of natural product and treatment of different diseases being accepted globally in process of new drug development (Hughes *et al*., 2011). As risk(s) of toxicological manifestations with natural products and nutraceuticals are there, hence, while developing natural products, it's imperative to screen the test moieties' for its safety beside its efficacy for treatment of different ailments (Mohd and Jyoti, 2012, Steinmetz and Spack, 2009).

Rosa moschata belongs to family Rosaceae, which is famous for its aesthetics and medicinal properties (Barkatullah and Ibrar, 2011, Bobita *et al*., 2012, Sharma and Devi, 2013). Species of rose are known for aesthetic and medicinal properties. Rosa genus have approximately 120 species. Its local names are Zangley Gulap", "Kurach"or "Qorach". *Rosa moschata* is very famous for its medicinal purposes (Ajmal *et al*., 2012). Traditionally, it is used for ophthalmological disorders, diarrhea and inflammation (Akhtar *et al*., 2013, Haider *et al*., 2011). It is also used in different herbal product like Gulkand, which is used for laxative and general tonic purposes (Khan *et al*., 2013). *Rosa moschata* contains Palmitic acid, stearic acid, linoleic acid, oleic acid, margaric acid and Linolenic acid, (Bobita *et al*., 2012). The species is rich with vitamin A, vitamin C, vitamin E and flavonoids (Honarvar *et al*., 2011). Essential oil of *Rosa moschata* is reported for its antioxidant properties (Speisky *et al*., 2006). Genus Rosa is rich with phenolic compounds and other phytochemicals that have antioxidant activity (Khurshid *et al*., 2018, Ouerghemmi *et al*., 2020). More. *Rosa moschata* has been reported to have antioxidant activity (Ouerghemmi *et al*., 2016, Ishaque *et al*., 2017). The plant has been effective in treatment of stress as per our recent report (Jamal *et al*., 2019).

We have recently reported that crude methanolic extract of fruits of *Rosa moschata* has antispasmodic activity, hence, the current work is carried out to know that in which fraction the relaxant moiety is concentrated. The current work will help that scientists for targeting the relaxant fraction for subsequent isolation of relaxant moiety (Ali *et al*., 2014).

MATERIALS AND METHODS

The study was carried out at Department of Pharmacology, IBMS Khyber Medical University Peshawar. The study protocol was approved by Advanced Study & Research Board of Khyber Medical University (DIR/KMU-AS&RB/CP000544). Briefly describing, fresh fruits of *Rosa moschata* were collected from Dir Lower Sayar valley. *Rosa moschata* was identified by Professor Dr. Jehandar Shah, ex Vice Chancellor University of Malakand, Chakdara Dir. Fruits were dried under shade. Plant extracts was prepared using Commercial grade methanol (80%) as menstruum to prepare the extract from fruits. The extract was subjected

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to evaporation till a semisolid material was obtained. This was then fractionated with different grade solvents in increase order of polarity i.e. *n*-hexane > chloroform > ethyl acetate > *n*-butanol > aqueous fraction) (Ali *et al*., 2013, Ali, 2013). The fractions were concentrated using rotary evaporator at 36-42°C till became semi solid. The solutions were prepared on the same day of experiments in distilled water.

Drug and standards

Throughout the experiments, analytical grade chemicals were used. From BDH, Poole, England acetylcholine was purchased, which was used in quiescent doses for the maintenance of tissues. All other chemicals were of E Merck grade. Verapamil was purchased from Sigma.

Animals

Under controlled environment of 25±2°C, 40 local breed's rabbits (weighing 1.5-2 kg) of both sexes were housed at the animal house of the Institute of Basic Medical Science, Khyber Medical University Peshawar, Pakistan. The animals were fasted overnight. The study protocol was approved by Ethics Board of the Advanced Study & Research Board of Khyber Medical University vide reference No.DIR/KMU-AS&RB/ CP000544.

Effects of different fractions of methanolic extract of Rosa moschata on spontaneous and KCl-induced rabbits' jejunal preparations

The antispasmodic profile of the different fractions of *Rosa moschata* was carried out on jejunal preparations of rabbits. Following cervical dislocation of rabbits, abdomens of the rabbits were opened, and jejunum was isolated. The jejunum was kept in Tyrode's solution aerated carbogen gas. 1.5-2cm tissues were suspended in organ bath containing Tyrode's solution. Bath temperature was 37±1°C throughout the experiments. Acetylcholine was used to verify normal tissue responses. Possible effects of test samples of *Rosa moschata* in concentrations 0.01, 0, 03, 0.1, 0.3, 1, 3, 5 and 10 mg/ml were assessed on spontaneous activity of rabbit's jejunal preparations. In similar fashion, the tests samples were screened on 80 Mm KCl induced contractions (Ali, 2013, Ali *et al*., 2014, Ali *et al*., 2013) as possible relaxant constituents relaxed the 80 Mm KCl induced contractions.

Effects of different sub fraction of fruit of Rosa moschata on calcium concentration response curves

To investigate for its possible mechanisms for relaxant activity, calcium chloride curves were constructed as per protocols in vogue in our laboratory (Ali, 2013, Ali *et al*., 2014, Ali *et al*., 2013). For confirmation of relaxant activity of test samples, we constructed calcium constructed calcium concentration response curves (CCRCs). This was done in the presence and absence of verapamil and test samples. Briefly describing, first the jejunal preparations were stabilized in Tyrode's solution for about 30 minutes. Then the tissues were decalcified through washing with Potassium Normal Tyrode's solution and Potassium Rich Tyrode's solutions for 2-5 times respectively. Standard control calcium curves were constructed using concentration in range of (1- 256)×10^{-4} M of calcium chloride. Tissues were treated with different concentration of 0.1- 10 mg/ml. Following an incubation time of 45 minutes, Calcium concentration response curves (CCRCs) were constructed using calcium chloride. Similarly, calcium curves were constructed in presence of verapamil (standard calcium channel blocker). Their EC_{50} values were noted and checked for possible right shift.

STATISTICAL ANALYSIS

Intestinal responses were expressed as % of control maximum response. All mean values of intestinal responses on its respective concentrations i.e. 0.01, 0.03, 0.1, 1.0, 3.0, 5.0 and 10 mg/ml were plotted as dose response curves. For CCRCs, calcium concentrations were plotted on x axis versus its respective responses on y-axis. The level of significances was tested in Graph Pad Prism 6 on 95% CI, P value less than 0.05 using One-way ANOVA.

RESULTS

Results of test fractions on spontaneous and KCl-induced contractions are presented in fig. 1(A, B, C, D, E). EC_{50} values for Rm. *n*-hex on spontaneous and KCl-induced contractions were 4.17±0.22 and 12.05±1.1mg/ml, respectively. Similarly, results for EC_{50} values of Rm. $ChCI_3$ on spontaneous and KCl-induced contractions were 3.65±0.16 and 3.93±0.27 mg/ml, respectively. For Rm. EtOA, its respective EC_{50} values were 0.812±0.149 and 2.01±0.08 mg/ml.

For Rm. *n*-BuOH fraction, its EC_{50} values for spontaneous and KCl induced values were 0.84±0.09 and 2.13±0.02 mg/ml. For Rm. Aqueous fraction, the EC_{50} values for both spontaneous and KCl-induced contractions were 5.89± 0.12 and 5.89±0.183 mg/ml, respectively.

Similarly, Fig. 2 is plotted to show the effects of different fractions of *Rosa moschata* on CCRCs with a possible right shift in its EC_{50} values expressed as log $[Ca^{++}]$M values. Log $[Ca^{++}]$M EC_{50} value for Rm.*n*-hex (3mg/ml) was -1.63±0.01 *vs* control with EC_{50} -2.68±0.007. While Rm-$ChCI_3$ (0.3 mg/ml) observed an EC_{50} value -1.61± 0.00 *vs* control EC_{50} -2.78±0.00.

In presence of 0.3mg/ml EtOA fraction, its EC_{50} value was -2.22±0.035 *vs* control EC_{50} -2.71±0.21. For *n*-BuOH fraction, EC_{50} value was -1.82±0.00 *vs* control of EC_{50} - 2.28± 0.049 at concentration of 0.3 mg/ml. While the rightward shift produced by Rm. Observed EC_{50} value for aqueous fraction Rm. Aq (3mg/ml) was -1.98±0.014 *vs* control of EC_{50} -2.15±0.007.

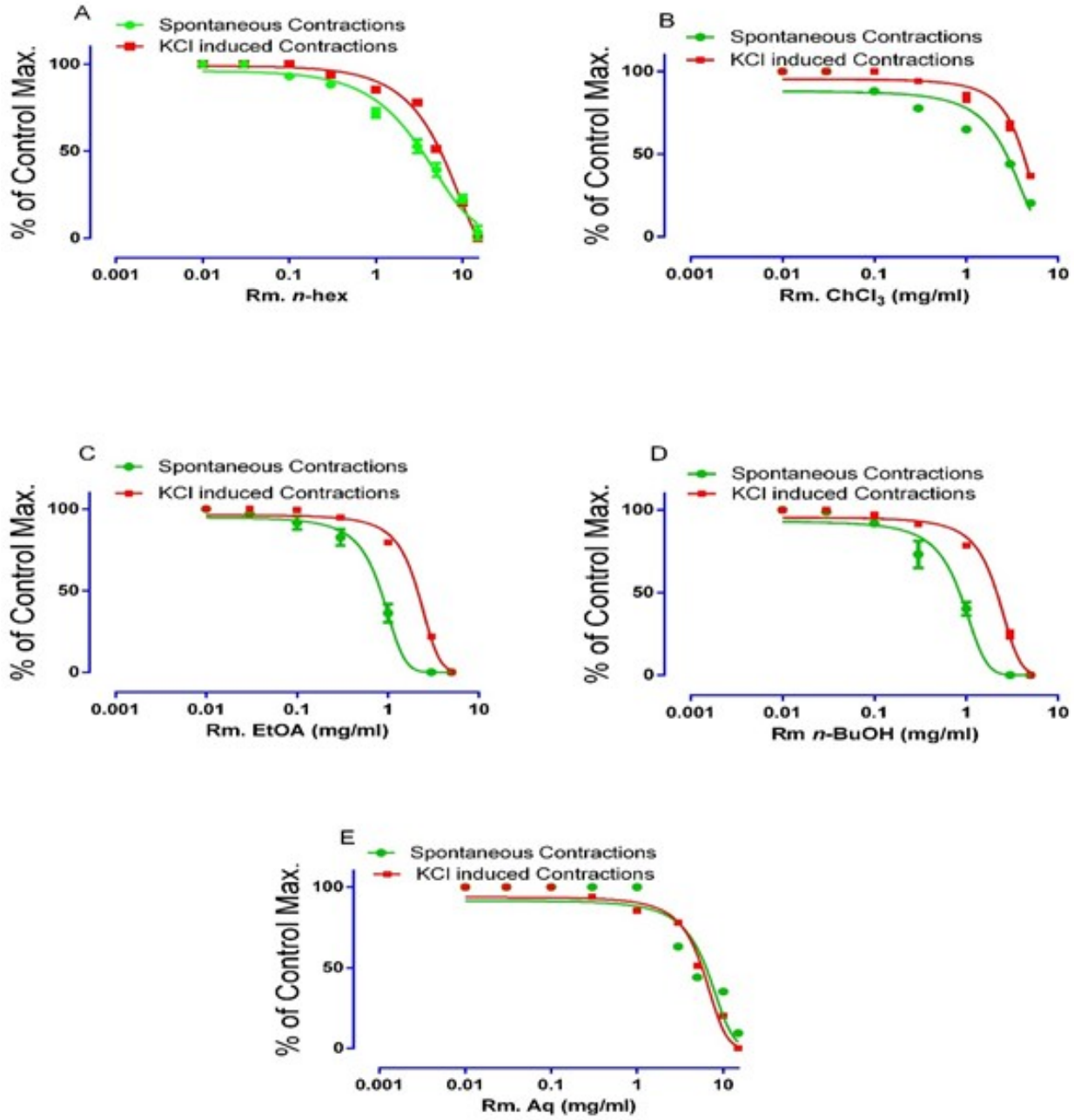

Fig. 1: Graphical presentation to show effects of different fractions (A,B,C,D,E) of fruits of *Rosa moschata* on spontaneous and KCl-induced contractions in rabbits' jejunal preparations (*n*=4).

DISCUSSION

In our previous work, we have reported that *Rosa moschata* contains flavonoids, tannins, saponins, phenolic contents, carbohydrates, terpenoids, and proteins (Ali *et al*., 2014). The relaxant effects may be attributed to these contents as flavonoids and terpenes, in general have antispasmodic activity. The current work confirms our previous report about antispasmodic activity of *Rosa moschata* as relaxant activity is found in different fractions of *Rosa moschata* this time. The contractile responses of intestinal smooth muscles are due to its periodic depolarization and repolarization. Calcium plays vital role in depolarization phase. It is the cytosolic calcium level that helps in subsequent proteins phosphorylation and helps in its contractions (Karaki and Weiss, 1988). The calcium may go through different mechanisms for example either through the entrance of calcium from extra cellular source (Farre *et al*., 1991) or through the release of calcium from intracellular stores (Fleckenstein, 1977). Explaining the mode of action for relaxing effect, different fractions of *Rosa moschata*

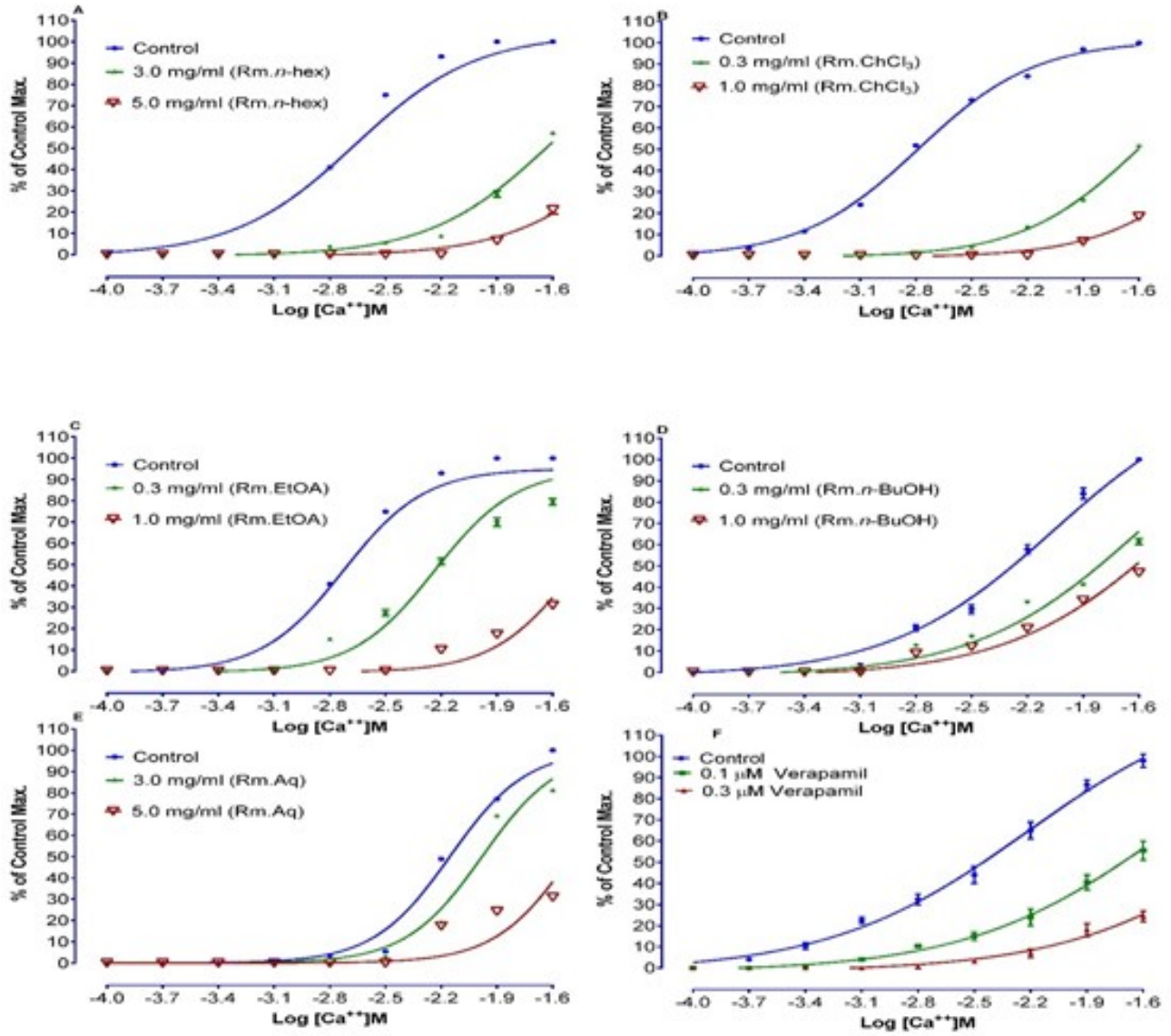

Fig. 2: Calcium chloride curves in the presence and absence of different fractions (A, B, C, D) of *Rosa moschata* and Calcium chloride curves in the presence and absence of verapamil. (Values represent the mean ± SEM, n = 4).

relaxed the KCl-induced contractions. Relaxing effect on KCl induced contractions implies for involving of calcium channels inhibition, yet it needs further confirmation through construction of CCRCs in the presence and absence of test samples as well as standard verapamil for easy reference. Thus, the relaxing effects of test samples of *Rosa moschata* may be attributed to involvement of inhibition of calcium channels (Fleckenstein, 1977). This is evident from the fractions respective EC_{50} values for its effects on spontaneous activities as well from relaxing effects on 80mM KCl-induced contractions. Studies have shown that relaxation on 80 mM KCl induced contractions are suggesting for involvement of calcium channel blocking mechanisms. But it is also a fact that all relaxant effects on contractions induced by KCl do not always suggest for blocking of calcium channels (Kobayashi *et al*., 1989). Thus, we plotted the CCRCs for confirmation of its mechanism (Farre *et al*., 1991). Calcium enters from extra-cellular spaces into the cytosol of cells through voltage gated calcium channels. This leads to depolarization of the tissues. As all test fractions of *Rosa moschata* produced right shift in EC_{50} values in manner resembling the effect of verapamil (Fleckenstein, 1977, Cortes *et al*., 2006, Ali *et al*., 2011b); therefore, we concluded that the spasmolytic effect is through the blockade of calcium channels (Farre *et al*., 1991). Activity guided fractionation proved that spasmolytic components were concentrated in different fractions with order of spasmolytic potency based on EC_{50} values as ethyl acetate > chloroform

>butanol> n-hexane > aqueous fraction. The relaxant effects may be attributed to the contents such as flavonoids and terpenoids that have been previously reported to have antispasmodic activity (Ali *et al*., 2011, Ali, 2013, Gilani *et al*., 2008).

CONCLUSION

Ethyl acetate fraction and chloroform fractions of *Rosa moschata* are potential target for activity guided isolation of calcium channel antagonists as these fractions relaxed the 80mM K-Cl induced contractions. Further investigations are required to isolate and characterize pure spasmolytic compound(s) from *Rosa moschata* fruits.

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