SPASMOGENIC AND SPASMOLYTIC ACTIVITIES OF *ONOSMA GRIFFITHII* VATKE

NIAZ ALI[1&3], BASHIR AHMAD[2] AND SYED WADOOD ALI SHAH[3]

[1]*Institute of Basic Medical Sciences, Khyber Medical University, KPK, Peshawar, Pakistan*
[2]*Centre of Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan*
[3]*Department of Pharmacy, University of Malakand, Chakdara, Dir (Lower), Khyber Pakhtunkhwa, Pakistan*

ABSTRACT

Methanolic extract of *Onosma griffithii* and its fractions were evaluated for possible effects on rabbits' jejunum preparations. Rabbits of either sex (weight 1.5-2.0 kg) were used in experiments. Studies were carried out on rabbits' jejunum preparations. Crude methanolic extract of *Onosma griffithii* (Meth.OG) was tried in concentrations of 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 mg/ml on rabbits' jejunum preparations. Meth.OG was also tried on KCl-induced contractions to explain its possible mode of actions in the presence and absence of atropine (0.03 µM). Fractions of Meth.OG were tried in similar manner. Calcium chloride curves were constructed for Meth.OG treated tissues that were compared with curves constructed for verapamil in same fashion. Preliminary phytochemical screening of the plant was also performed. Meth.OG increased the amplitude of spontaneous activity of rabbits' jejunum preparations at concentrations of 0.1, 0.3 and 1.0 mg/ml. However, spasmolytic effects were observed at higher concentrations 3.0, 5.0 and 10.0 mg/ml. Mean EC_{50} values (mg/ml), respectively, in absence and presence of atropine were 7.5 ± 0.25 (6.9-8.4, n=6) and 3.0 ± 0.17 (2.3-3.5, n=6, p<0.05). Mean EC_{50} values, respectively, for effects on spontaneous and KCl-induced contractions were 7.5 ± 0.25 (6.9-8.4, n=6) and 7.3 ± 0.35 (6.25-8.2, n=6, p<0.05). n-Hexane, chloroform and ethyl acetate fractions showed their respective EC_{50} values (mg/ml) 9.7 ± 0.25 (8.6-10.2, n=6), 4.0 ± 0.2 (3.5-4.6, n=6) and 1.07 ± 0.093 (0.78-1.5, n=6). EC_{50} values for calcium chloride curves in presence of 0.3 mg/ml Meth.OG were – 2.27 ± 0.038 (– 2.4 to – 2.10, n=6) vs. control – 2.78 ±0.04 (–2.9 to – 2.6, n=6, p<0.05) Log $[Ca^{++}]$M. Comparing with curves of calcium chloride constructed in presence of 0.1 µM verapamil, the EC_{50} (log $[Ca^{++}]$ M) values were – 1.82 ± 0.087 (– 2.0 to – 1.65, n=6) vs. control – 2.64 ± 0.089 (– 2.9 to – 2.4, n=6) demonstrated a right shift (p<0.05). Meth.OG tested positive for terpenes, saponins, sterols, flavonoids and carbohydrates. We concluded that the relaxant effect of Meth.OG is exerted through blocking of calcium channels. However, n-butanolic and aqueous fractions produced spasmogenic effects that require further work for isolation of pharmacologically active substances.

Keywords: *Onosma griffithii*; spasmolytic, spasmogenic, calcium antagonists, EC_{50}.

INTRODUCTION

Onosma griffithii belongs to family boraginaceae that consists of about 100 genera and 2000 species that are widely distributed in tropical regions of the world (Ali and Nasir, 1983). Antiviral, antibacterial, antioxidant and anti-inflammatory activities of some of species of *Onosma* have been reported (Bashir *et al*., 2009a; Tosun *et al*., 2008). Traditional uses of *Onosma* are in the management of bladder pain, rheumatism, kidney pain and palpitation of heart (Ahmad, 2005). Coloring property and cholinesterase inhibitory activity of *Onosma hispidum* have been documented (Shahina, 2005; Ahmad *et al*., 2003). Reported compounds from *Onosma hispidum* are 2-[(4-methylbenzyl)amino]benzoic acid (Onosmin A) and Me 2-[(4-methylbenzyl)amino]benzoate (Onosmin B), apigenin, 6,4'-dimethoxy-3,5,7-trihydroxyflavone, 6,7-dimethoxy-3,5,4'-trihydroxyflavone and apigenin 7-O-?-D-glucoside (Ahmad *et al.*, 2005). Roots of boraginaceae contain pharmacologically active substances like shikanins and alkanins (Ufuk *et al*., 2004). Antioxidant and antimicrobial activities of *Onosma argenatum* and *Onosma echioides* have been reported. *Onosma echioides* has shown antioxidant, antitumor and antiproliferative effects that may be attributed to two major constituents i.e. alkannins and shikonins (Sharma *et al.*, 2004). Onosmone, a new ketone, and bauerenone, a known anticancer triterpenoid have been isolated from the chlroroform fraction of leaves of *Onosma limitaneum* (Ahmad *et al*., 2005). Thus the genus *Onosma* is of great pharmacological importance. Little is known about the pharmacological activities of *Onosma griffithii*. So far, we have reported parasiticidal, antibacterial and antifungal activities of *Onosma griffithii* (Bashir *et al*., 2009a). Our current work describes the effects of Meth.OG and its fractions on rabbits' jejunum preparations to explain its antispasmodic use scientifically.

MATERIALS AND METHODS

Collection and authentication of plant materials

Whole plant material was collected in June-July, 2005 from the hills of Kabal and Shamuzai regions of Swat. Professor Dr. Jehandar Shah authenticated the plant that was deposited with voucher specimen OG-05 to Malakand University.

**Corresponding author:* e-mail: niazpharmacist@yahoo.com

Extract preparation and fractionation

Shade dried powdered parts of whole plant of *Onosma griffithii* (7 kg) were macerated in methanol (90-95%) and occasionally shaked on alternate day. After fifteen days, the materials were filtered off and the process was repeated thrice. All the filtrates were combined and subjected to evaporation using a rotary evaporator at 40° C till a black greenish extract (175 g) was obtained. Meth.OG (150 g) was fractionated, successively, with *n*-hexane, chloroform, ethyl acetate and *n*-butanol yielding 30g, 20g, 2g, 2g and 65 g aqueous respectively.

Drugs and animals

We used analytical grade chemicals in the experimentations. Acetylcholine (ACh) (BDH Chemicals, Poole, England), and all other chemicals of E. Merck Germany were used. Fresh solutions were made at the time of experiments. New Zealand rabbits weighing about 1.5-2.0 kg were utilized in the experiments. The animals were dealt as per the "Animals Bylaws, 2008 of the University of Malakand (Scientific Procedures Issue-1)" complying with international standards for dealing the experimental animals. They had free access to tape water. However, they were kept in fasting conditions for 24 hours before the start of experiments.

Data recording, interpretation of graph tracings and statistical analysis

MLT 0210/A Force Transducer, connected with Power lab was used to record the data. Chart setting parameters were 5Hz X 10 gain, Low pass, range 20 mv and rate 40/S. Graph tracings were interpreted with Chart 5 program. Data were expressed as mean ± standard error of mean (S.E.M). Student "t" test was used for analysis of various parameters. *p* values less or equal to 0.05 was considered as statistically significant.

Preliminary phytochemical screening

Meth.OG was tested for presence of different phytochemicals like terpenes, saponins, alkaloids, tannins, flavonoids, carbohydrates and sterols (Aduragbenro *et al.*, 2009; Harborne, 1973; Kokate *et al*., 1994).

Jejunum's preparations

Meth.OG was tested at concentrations of 0.01, 0.03, 0.1, 0.3, 3.0, 5.0 and 10.0 mg/ml (Gilani *et al*., 2005; Niaz *et al*., 2009). Rabbits were sacrificed and their abdomens were opened. Jejunum was removed and placed in petri dish containing aerated Tyrode's solution. Pieces of about 1.5-2 cm were mounted in 10 ml organ bath aerated with carbogen gas. Experiments were carried out at 37 ± 1°C. When the tissues were stabilized for 30 minutes, Meth.OG was tried at the said concentrations for possible effects on spontaneous jejunum preparations at a minute interval.

Effects of Meth.OG on contractions induced by KCl

Contractions in rabbits' jejunum preparations were produced by 80 mM KCl. Meth.OG was tried in similar concentrations of 0.01, 0.03, 0.1, 0.3, 3.0, 5.0 and 10.0 mg/ml (Gilani *et al*., 2005; Niaz *et al*., 2009). The effects were noted. In similar way, pharmacological activity guided fractionation was performed and screened for activity, thereafter. The spasmogenic effects of fractions were quantified against acetylcholine maximum (1.0 µM), a standard spasmogenic agent (Bashir *et al*., 2009b); whereas the relaxant effect was expressed as percent of control maximum.

Calcium channel blocking activity

Initially the tissues were stabilized in Tyrode's solution at least for 30 minutes. Then the tissues were exposed to K^+-Normal Tyrode's solution and K^+-Rich Tyrode's solution, thereafter (Gilani *et al*., 2005) that lead to decalcification of tissues. Calcification of the tissues were performed in a range of 1×10^{-4} – 256×10^{-4} Molar concentrations of calcium. Meth.OG at concentration 0.3 mg/ml and 1.0 mg/ml was added to the tissues and incubated for one hour. Again the calcium chloride was added in concentration of 1×10^{-4} – 256×10^{-4} and curves were constructed in the presence of Meth.OG. Similar curves were constructed in the presence of 0.1 and 0.3 µM of verapamil. The curves were compared and checked for right shift in EC_{50} values.

RESULTS

Preliminary phytochemical screenings

Meth.OG tested positive for terpenes, saponins, sterols, flavonoids and carbohydrates.

Effects on rabbits' jejunum preparations and on KCl-induced contractions in rabbits' jejunum preparations

Fig. 1 describes the effects of Meth.OG in the presence and absence of atropine on spontaneous rabbits' jejunum preparations. In the absence of atropine, gradual increase in amplitude of spontaneous activity was observed with maximum spasmogenic effect (18.75±3.4 % of control max.) at concentration 1.0 mg/ml. In the presence of atropine, there was no spasmogenic effect, and it is deduced that the spasmogenic effects are through the cholinergic muscarinic receptors. Left shift in mean EC_{50} values (mg/ml), respectively, in absence and presence of atropine are 7.5 ± 0.25 (6.9-8.4, n=6) and 3.0 ± 0.17 (2.3-3.5, n=6, $p < 0.05$) confirmed that the spasmogenic activity is via cholinergic muscarinic receptors. At a concentration of 3 mg/ml and onward, spasmolytic effects were observed with maximum response at concentration 10 mg/ml. Fig. 2 describes the effects of Meth.OG on contractions induced by 80 mM KCl. Mean EC_{50} values (mg/ml), respectively, for effects on spontaneous and KCl-induced contractions are 7.5 ± 0.25 (6.9-8.4, n=6) and 7.3 ± 0.35 (6.25-8.2, n=6) non-significant (p 0.05). Similar EC_{50} values of the fig. 2 suggest that relaxation may be through same or single mechanism that requires further work. Results of *n*-hexane, chloroform and ethyl

acetate fraction at similar test concentrations are expressed in fig. 3 with their respective EC_{50} values (mg/ml) 9.7 ± 0.25 (8.6-10.2, *n*=6), 4.0 ± 0.2 (3.5- 4.6, *n*=6) and 1.07 ± 0.093 (0.78-1.5, *n*=6). Results of *n*-butanol and aqueous fraction are plotted in fig. 4 as percent of control maximum. Maximum spasmogenic effect at 10.0 mg/ml of Meth.OG, respectively, for aqueous and *n*-butanol fraction is 235 ± 5% and 150 ± 5.5% (p <0.05). The spasmogenic effects were standardized against 1.0 µM ACh maximum and are expressed in fig. 5. At concentration 5 and 10 mg/ml, maximum responses (% of ACh max.) for aqueous, *n*-butanol fractions were 72 ± 5.9%, 8.6 ± 1.4% and 90.4 ± 8, 72 ± 5.9 %, respectively.

Calcium channel blocking activity

Relaxant effect on contractions induced by KCl does not suggest that the mechanism is through the calcium channels, hence, results of calcium chloride curves both for the Meth.OG and verapamil treated tissues are plotted vs. control in fig. 6. According to fig. 6A, EC_{50} values (log [ca^{++}]M) for 0.3 mg/ml treated calcium chloride curves is – 2.27 ± 0.038 (– 2.4 to – 2.10, *n*=6) vs. control – 2.78 (–2.9 to – 2.6, *n*=6). In the presence of Meth.OG (0.3 mg/ml), the calcium chloride could only produce non parallel maximum response 68.02 ± 9 % of control response at – 1.6 Log [Ca^{++}] M and 27.47 ± 4.7 % of control in the presence of Meth.OG 1.0 mg/ml. Likely, verapamil, a standard calcium channel blocker, at concentration 0.1 µM shifted the curves to right with EC_{50} (log [Ca^{++}]M) values – 1.82 ± 0.087 (– 2.0 to – 1.65, *n*=6) vs. control – 2.64 ± 0.089 (– 2.9 to – 2.4, *n*=6) (fig. 6B).

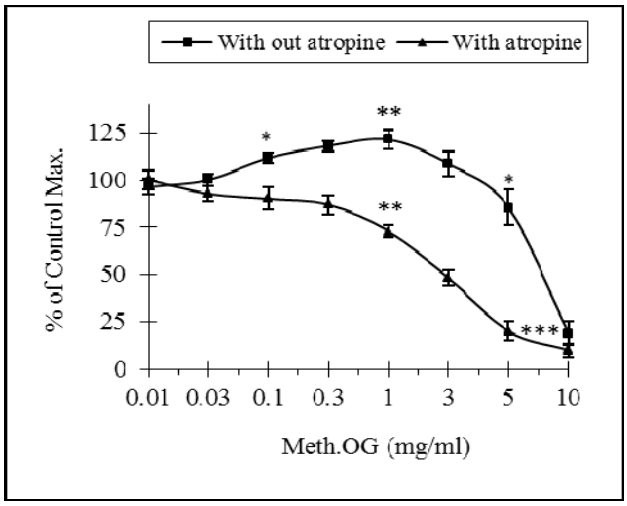

Fig. 1: Effects of Meth.OG on spontaneous rabbits' jejunum preparations in presence and absence of atropine (0.03 µM). (Mean ± SEM, *n*=6,*p<0.05,**p<0.01,***p<0.001 *vs*. control max.)

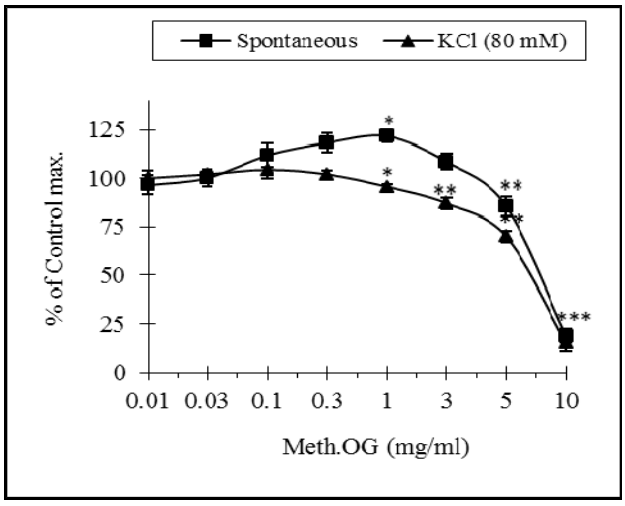

Fig. 2: Effects of Meth.OG on spontaneous and KCl-induced contractions of rabbits' jejunum preparations. (Mean ± SEM, *n*=6, *p<0.05, **p<0.01, ***p<0.001 *vs*. control max.).

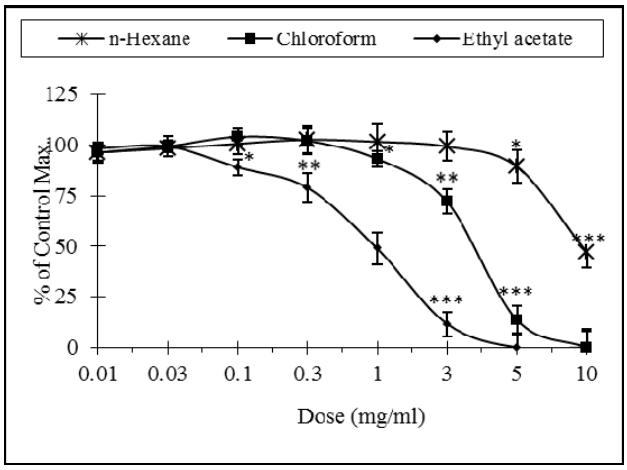

Fig. 3: Effects of fractions of Meth.OG that relaxed rabbits' jejunum preparations.
(Mean ± SEM, *n*=6, *p<0.05, **p<0.01, ***p<0.001 *vs*. control max.)

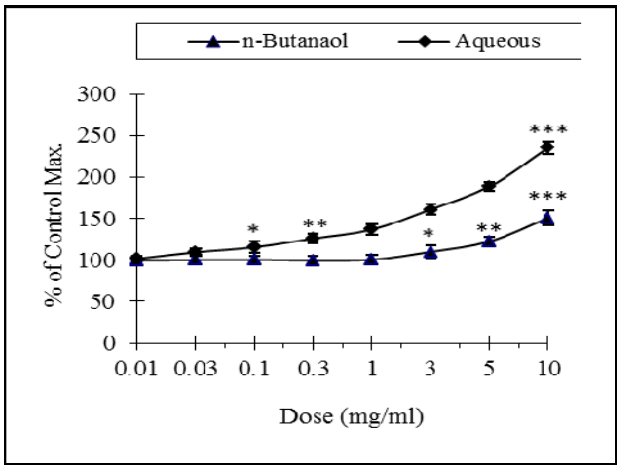

Fig. 4: Spasmogenic effects of *n*-butanolic and aqueous fractions of Meth.OG.
(Mean ± SEM, *n*=6, *p<0.05, **p<0.01, ***p<0.001 *vs*. control max.)

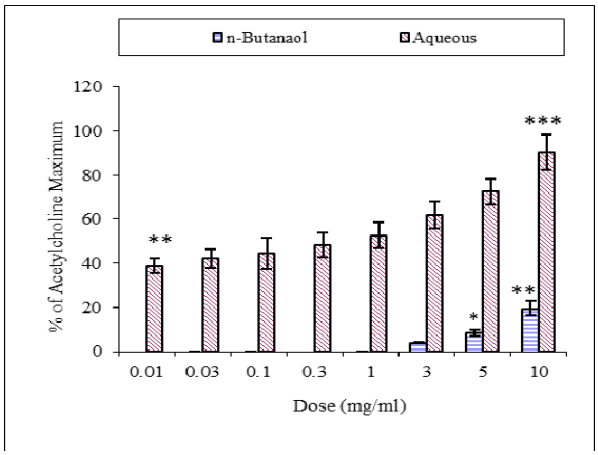

Fig. 5: Quantified spasmogenic effects (% of ACh max.) of *n*-butanolic and aqueous fractions of Meth.OG (Mean ± SEM, *n*=6, **p* <0.05, ** *p* <0.01, *** *p* <0.001 *vs*. acetylcholine max.)

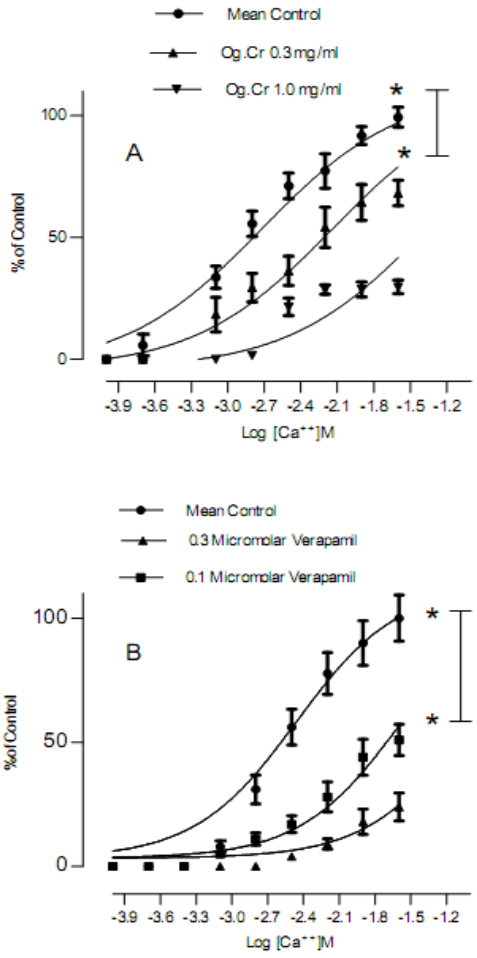

Fig. 6: A. Effects of Meth.OG on calcium chloride curves *vs*. mean control to show the right shift in EC_{50} values (**p* <0.05). B. Effects of Verapamil on calcium chloride curves *vs*. mean control to show the right shift in EC_{50} values (**p* <0.05).

DISCUSSION

As Meth.OG tested positive for the presence of terpenes, saponins, sterols and flavonoids, therefore, the activity may be attributed to these phytochemicals. Gradual increase in amplitude of spontaneous activity of rabbit's jejunum preparations was observed with maximum spasmogenic effect (18.75 ± 3.4%) at concentration 1.0 mg/ml. In the presence of atropine, a cholinergic antagonist, there was no spasmogenic effect pointing that the spasmogenic effects are through the cholinergic muscarinic receptors (Bashir *et al*., 2009b; Wadood *et al*., 2010). The left shift in the mean EC_{50} values (mg/ml), respectively, in the absence and presence of atropine 7.5 ± 0.25 (6.9-8.4, *n*=6) and 3.0 ± 0.17 (2.3-3.5, *n*=6) confirmed that the cholinergic receptors are involved in spasmogenic effects (Gilani *et al*., 2005; Bashir *et al*., 2009b). It is an established fact that the contractile proteins use cytosolic calcium for spasmogenic response. Whereas, the cytosolic calcium is regulated by different sources of calcium, therefore, different mechanisms may be involved in the spasmogenic activity. Like the entrance of calcium from extracellular source (Nasu and Magami, 2000; Nasu, *et al.*, 2001), and release of calcium from intracellular stores (Karaki and Mitsui, 1988). Further, acetylcholine is a natural spasmogen that increases intracellular calcium through cell membrane, by the release of calcium from internal stores like cytoplasmic reticulum and from the calcisomes; and of course through the inositol triphosphate (IP3) dependent pathway (Souza and Aka, 2006). Similarly, as reported by Gillespie and Mackenna (1960), involvement of nicotinic receptors for spasmogenic activity cannot be ruled out in intestinal preparations (*in vitro*). According to them, nicotine produces contraction due to stimulation of cholinergic neurons of the parasympathetic division of autonomic nervous system that innervates the intestine (Gillespie and Mackenna, 1960). As the left shift in the mean EC_{50} values confirmed that cholinergic muscarinic receptors are involved (Bashir *et al*., 2009b; Wadood *et al*., 2010). Hence, further work is required to elucidate for possible other mechanisms that are responsible for the spasmogenic effect as discussed. Activities directed fractionation of the Meth.OG revealed that the spasmogenic effect was concentrated in *n*-butanol and aqueous fraction. Quantifying the spasmogenic effects as percent of ACh maximum (1.0µM), aqueous fraction produced predominant spasmogenic effect than *n*-butanol. Therefore, *n*-butanol and aqueous fractions could be a source for spasmogens of *Onosma griffithii* that require further work for its identification and subsequent isolation. At a concentration of 3.0 mg/ml and onward, spasmolytic effects were observed with maximum response at concentration 10 mg/ml. Explaining the possible mode of action for spasmolysis, the Meth.OG on KCl-induced contractions also produced relaxant effects with more or less similar mean EC_{50} values for effects on

spontaneous activities, suggesting that relaxation may be through calcium channel blocking mechanism. As all relaxant effects on contractions induced by KCl do not suggest that the effects is through blocking of calcium channels (Kobayashi *et al*., 1989), therefore, we plotted the calcium chloride curves that helped us for confirmation of its mechanism (Niaz *et al*., 2009). Calcium exchanges through voltage operated calcium channels from extra-cellular spaces into the cytosol of cells. This lead to depolarization of the tissues (Ghayur and Gilani, 2005; Gilani *et al*., 2005). Since Meth.OG produced similar effects with a right shift in EC_{50} values like the right shift of verapamil (Fleckenstein, 1997; Cortes *et al*., 2006); therefore, we concluded that the spasmolytic effects is through the blockade of calcium channels. Activity guided fractionation proved that spasmolytic components were concentrated in different fractions with order of spasmolytic potency as ethyl acetate > chloroform > *n*-hexane. The spasmolytic activity may be attributed to the phytochemicals such as saponins, flavonoids, sterols, tannins and triterpenes as similar type of studies in medicinal plants have been reported to have relaxant activities (Niaz and Wadood, 2011, Cortes *et al.*, 2006). The spasmogens were concentrated in *n*-butanol and aqueous fractions.

CONCLUSION

The results confirm that relaxant effect of *Onosma griffithii* on rabbits' jejunum preparations is through calcium channel blocking mechanism because of the calcium antagonists that concentrated in ethyl acetate, chloformic and n-hexane fractions. Spasmogenic effects were concentrated in *n*-butanol and aqueous fractions. Further work is required to isolate the pharmacologically active phytochemical(s).

ACKNOWLEDGMENT

The author wants to thank the Higher Education Commission of Pakistan for Research Grant No: 1-23/HEC/R and D- 2007/CASE/34-Local.

REFERENCES

Aduragbenro DA, Yeside OO, Adeolu AA, Olanrewaju MJ, Ayotunde SA, Olumayokun AO and Janet MM (2009). Blood pressure lowering effect of *Adenantherapavonina* Seed extract on normotensive rats. *Rec. Nat. Prod.,* **3**: 282-289.

Ali SI and Nasir YJ (1983). *Onosma griffithii* Vatke (Boraginaceae) (Eds.) *In*: Flora of Pakistan, Vol.191, Shamim Printing Press, Karachi p.103.

Ahmad I, Anis I, Malik A, Nawaz SA and Choudhary MI (2003). Cholinesterase Inhibitory Constituents from *Onosma hispida*. *Chem. Pharm. Bull.,* **51**(4): 412-414.

Ahmad I, Nawaz SA, Afza, N, Malik A, Fatima I, Sher Bahadar K, Ahmad M and Choudhary M Iqbal (2005). Isolation of Onosmins A and B, Lipoxygenase Inhibitors from *Onosma hispida*. *Chem. Pharm. Bull.,* **53**(8): 907-910.

Ahmad VU, Kousar F, Khan A, Zubair M, Iqbal S and Tareen RB (2005). A New Ketone and a known anticancer triterpenoid from the leaves of *Onosma limitaneum*. *Helv. Chim. Acta*., **88**(2): 309-311.

Bashir A, Niaz A, Shumaila B, Choudhary MI, Sadiq A and Ibrar K (2009a). Parasiticidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. *Afr. J. Biotechnol.,* **8**(19): 5084-5087.

Bashir A, Niaz A, Shumaila B, Jamshaid A, Sadiq A and Ibrar K (2009b). Cholinomimetic and calcium channel blocking activity of *Tylophora hirsuta* Wall. *J. Chem. Soc. Pak.,* **31**(4): 647-651.

Cortes AR, Delgadillo AJ, Hurtado M, Dominguez-Ramirez AM, Medina JR and Aoki K (2006). The Antispasmodic Activity of *Buddleja scordioides* and *Buddleja Perfoliata* on isolated intestinal preparations. *Biol. Pharm. Bull*., **29**(6): 1186-1190.

Edeoga HO, Okwu DE and Mbebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.,* **4**: 685-688.

Fleckenstein A (1977). Specific pharmacology of Ca^{++} in myocardium, cardiac pacemakers and vascular smooth muscle. *Rev. Pharmacol. Toxicol.,* **17**: 149-166.

Ghayur MN and Gilani AH (2005). Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. *Dig. Dis. Sci*., **50**(10): 1889-1897.

Gilani AH, Bukhari IA, Khan RA, Khan A, Ullah F and Ahmad VU (2005). Cholinomimetic and calcium channel blocking activities of *Carthamus oxycantha*. *Phytother.Res.,* **19**: 679-683.

Gillespie JS and Mackenna BR (1960). The inhibitory action of nicotine on the rabbit colon. *J. Physiol.,* **152**: 191-205.

Harborne JB (1973). Phytochemical methods. Chapman and Hall, London, p.117.

Karaki H and Mitsui M (1988). Verapamil-sensitive and less sensitive contractions in the intestinal smooth muscle of guinea pig *Taenia caeci*. *Jpn. J. Pharmacol*.46 : 325-330.

Kobayashi S, Kitazawa T, Somlyo AV and Somlyo AP (1989). Cytosolic heparin inhibits muscarinic and alpha-adrenergic Ca^{2+} release in smooth muscle. Physiological role of inositol 1,4,5-trisphosphate in lymphocytes. *Indian J. Nat. Prod.*, **23**(3): 8-12.

Kokate CK, Purohit A P and Gokhale SB (1994). Practical Pharmacognosy 2nd ed**.** Nirali Prakashan, Pune, Mumbai.

Nasu T and Magami G (2000). Actions of Mn^{2+} in the presence of ionophore A 23 187 in guinea-pig *Tænia coli*. *Pharmacol. Res.,* **41**(4): 397-403.

Nasu T, Toda H and Shibatah H (2001). Differential inhibitory mechanism of Fe^{+2} on contraction of ileal longitudinal smooth muscle. *Pharmacol. Res.,* **43**(1): 95-102.

Niaz A, Bashir A, Shumaila B, Jehandar S, Sadiq A and Manzoor A (2009). Calcium channel blocking activities of *Withania coagulans. Afr. J. Pharma. Pharmacol.*, **3(**9): 43.

Niaz A and Wadood AS (2011). Antispasmodic activity of *Teucrium stocksianum* Bioss. *Pak. J. Pharm. Sci.*, **24**(2): 171-174.

Wadood AS, Samir K, Waqar A and Niaz A (2010). Spasmogenic, spasmolytic and antihypertensive activity of *Forsskalea tenacissima* L. *Afr.J. Pharma. Pharmacol.*, **4**(6): 381-385.

Shahina N (2005). Structure and functionality of the pigments isolated from *Onosma hispidum* (ratanjot) *Terminalia catappa* (jangli badam) and other tropical plant. Ph.D. thesis, University of Karachi, Karachi.

Sharma S, Khan N and Sultana S (2004). Effect of *Onosma echioides* on DMBA/croton oil mediated carcinogenic response, hyperproliferation and oxidative damage in murine skin. *Life Sci.,* **75**(20): 2391-2410.

Souza A and Aka K J (2007). Spasmogenic effect of the aqueous extract of *Tamarindus indica* L. (caesalpiniaceae) on the contractile activity of guinea-pig *Taenia coli. Afr. J. Trad. CAM.*, **4**(3): 261-266.

Tosun A, Akkol EK, Bahadır O and Yeilada E (2008). Evaluation of anti-inflammatory and antinociceptive activities of some *Onosma* L. species growing in Turkey. *J. Ethnopharmacol*., **120**(3): 378-831.

Ufuk O, Maksut CK, Cavit K and Hasan SK (2004). Naphthoquinones from the Roots of *Onosma argentatum* Hub. (Boraginaceae). *Turk. J. Chem.*, **28**: 451-454.