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Myorelaxant and Spasmolytic Effects of *Globularia alypum* L. Extract on Rabbit Jejunum

A. Chokri, R. Doukali, K. El Abida and R. Ben Cheikh
Laboratoire des Molécules Bioactives, FST, Route d'Immouzzer,
B.P 2202, 30000 Fes, Morocco

Abstract: The aim of this study was to investigate the myorelaxant and the spasmolytic effects of the *Globularia alypum* L. (GA) extract and to elucidate its role on the Ca^{2+} mobilization. Contraction of isolated rabbit jejunum incubated in Tyrode solution was recorded in presence of methanolic extract of GA. This extract showed a reduction of the amplitude and the tone of spontaneous contraction in a concentration-dependent manner. Spasmolytic effect was studied on tonus increase induced by Ach 10^{-5} M or by Tyrode high KCl (100 mM). Acetylcholine (10^{-5} M) induced contraction was inhibited by cumulative concentrations of the extract. Also, GA completely relaxed the jejunum contracted by KCl. In order to assess if GA effect involved Ca^{2+} influx restriction, the jejunum was replaced in Ca^{2+} free Tyrode solution with EDTA (2 mM) or in Tyrode containing GA (6.4 mg mL^{-1}) and then was challenged with KCl or Ach. Pre-treatment with GA extract inhibited the restored spontaneous contraction obtained when Ca^{2+} was added to the bath. GA extract abolished tonic phase of Ach induced contraction and drastically diminished phasic one, while it completely abolished KCl induced contraction. Similar responses were obtained when the jejunum was placed in Tyrode Ca^{2+} free. Verapamil a standard spasmolytic agent or a sub maximal concentration of GA inhibited and right shifted the Ca^{2+} response curves realized on jejunum incubated in high K^+ Ca^{2+} free. All these results suggest that GA extract probably acts at least through a voltage-dependent Ca^{2+} channels blockade.

Key words: *Globularia alypum* L., myorelaxant, antispasmodic, Ca^{2+} blockade, smooth muscle

INTRODUCTION

Traditional pharmacopeia through the millennia often used plants. Indeed, it is now well reported that different plant extracts are used in different diseases (Radhika *et al*., 2010; Jothimanivannan *et al*., 2010; Kocyigit *et al*., 2010) and especially in gastrointestinal transit disorders (Thaina *et al*., 2008; Cortes *et al*., 2006; Bashir *et al*., 2006).

GA is a Globulariaceae and it is a shrub which grows on the Mediterranean basin (Essafi *et al*., 2006). Its height varies between 30 and 80 cm and the color of its flower goes from bleu to purple. In Morocco, it locally called Ain larneb and its leaves are used in traditional medicine as a hypoglycemic, a laxative, a purgative, a myorelaxant and an antispasmodic remedy (Ziyyat *et al*., 1997; Merzouki *et al*., 2000; Eddouks *et al*., 2002).

Some pharmacological activities of GA have been reported such as an antileucemic effect (Caldes *et al*., 1975) and an antioxydative one (Essafi *et al*., 2005). Skim *et al*. (1999) and Jouad *et al*. (2002) showed that oral administration of the aqueous extract of GA leaves produced a significant hypoglycaemic effect in normal and in hyperglycaemic rats. Recently, Zennaki *et al*. (2009) showed that methanolic extract of GA reduced remarkably glycemia, plasma lipids and has beneficial effects on redox status.

Some chemical investigations of aerial part of GA have been done and many constituents such as phenolic, phenylethanoid glycosides and flavonoid glycosides compounds (Essafi *et al*., 2005) and plenty of iridoids glycosides (globularioside) were isolated and their molecular formula determined (Essafi *et al*., 2006).

Despite the extensive use of GA for gastrointestinal problems such as intestinal spasms, it is difficult to correlate the biological effects with the traditional use, thus more research is required to scientifically prove the action and efficacy of GA.

In the other hand, the contractions of the smooth muscle including that of rabbit jejunum are dependent upon an increase in the cytosolic free Ca^{2+} levels (Karaki and Weiss, 1988; Karaki *et al*., 1997) and the entry via voltage-dependent Ca^{2+} channels is the one of the major mechanisms of Ca^{2+} influx for the

Corresponding Author: Rachid Bencheikh, Laboratoire des Molécules Bioactives, FST, Route d'Immouzzer, B.P 2202, 30000 Fes, Morocco Fax: 00212 535 608 214

initiation of smooth muscle contraction (Itoh *et al*., 1984; Goto *et al*., 1989; Shimizu *et al*., 2000).

It has been known that a solution high in K^+ elicits membrane depolarization and thus opens the voltage dependent Ca^{2+} channels to cause an influx of Ca^{2+} and finally induce muscle contraction (Godfraind *et al*., 1986; Horowitz *et al*., 1996; Ghayur and Gilani, 2004; Gilani *et al*., 2005; Thaina *et al*., 2008).

Also, it is well known that Ca^{2+} channel blockers as verapamil inhibit the Ca^{2+} influx into smooth muscle cells via voltage-dependent channels (Cortes *et al*., 2006; Eun *et al*., 2010; Beech *et al*., 1990; Zhou *et al*., 2010).

Since Ca^{2+} restriction influx has been often proposed as the origin of myorelaxant and spasmolytic effects of many medicinal plants (Fatehi *et al*., 2004; Estrada-Soto *et al*., 2007; Moazedi *et al*., 2007; Brankovic *et al*., 2009; Gilani *et al*., 2010), the present study was designed to see whether the spasmolytic activity of GA extract on isolated rabbit jejunum is due to possible Ca^{2+} channel blockade. Also, the present paper was conducted to validate GA popular use as spasmolytic remedy by people who remain without access to modern medicine.

MATERIALS AND METHODS

Plant material: GA has been collected in May 2008 and identified by the National Institute of Aromatic and Medicinal Plants (INPAM) where a voucher sample referenced under the No (MA-INP 80) was deposited. The leaves of the plant were washed with distilled water, air-dried and reduced to a powder. Ten grams of this powder were soaked in 100 ml of methanol for 24 h and concentrated on a rotary evaporator at 40°C (Ben Sassi *et al*., 2007), with a yield of 37%. The extract was then stored at -20°C.

Animal and tissue preparations: Rabbits of both sexes (1.8-2.5 kg) were kept in a standard environmental condition of humidity, temperature and light. The animals had free access to water and food until the experiment. However, the food was withdrawn 24 h prior the experiment. The rabbits were killed by exsanguination, segments of jejunum about 2-3 cm were quickly isolated and transferred in 50 mL organ bath chamber filled with Tyrode solution containing (in mM): 136 NaCl, 2.7 KCl, 1.4 $CaCl_2$ $2H_2O$, 0.5 $MgCl_2$ $6H_2O$, 11.9 $NaHCO_3$, 0.42 NaH_2PO_4 and 5.56 Glucose (bubbled continuously with 95 % O_2 -5% CO_2, pH 7.4 at 37°C) (Dar and Channa, 1999).

Isolated rabbit jejunum: The preparation was allowed to equilibrate for 30 min as the tissue exhibited stable spontaneous contraction. Segments of jejunum that did not show spontaneous contraction were discarded from the experimental protocol. No more than 3 experiments were realized on the same jejunum which always received the same extract.

Spasmolytic effect was studied on tonus increase induced by Ach 10^{-5} M and by Tyrode high KCl (100 mM). The latter was obtained by an equimolar replacement of NaCl by KCl (Parekh and Brading, 1991; Delaey *et al*., 2007).

In order to assess whether the effect of GA extract involves a Ca^{2+} channel blockade, the jejunum was replaced in Ca^{2+} free Tyrode solution with EDTA (2 mM) leading to the removal of Ca^{2+} from tissues. After the abolition of the spontaneous contraction, Ca^{2+} was added in a cumulative way in the absence or the presence of GA extract (6.4 mg mL^{-1}). In another series of experiments, the isolated rabbit jejunum were exposed for 5 min to Ca^{2+} free Tyrode's solution with EDTA (2 mM) and then to the high KCl (100 mM) Ca^{2+} free solution during 10 min. Controlled Ca^{2+} response curve was obtained with cumulative addition of $CaCl_2$ (0.1-20 mM). The same experiences were repeated in presence of sub-maximal concentration of GA (2.4 mg mL^{-1}) or verapamil (5.10^{-6} M), a standard voltage dependent Ca^{2+} channel blocker (Ohya *et al*., 1987; Terada *et al*., 1987).

Drugs: Ach, adrenalin and verapamil were purchased from Sigma Chemicals Co (St Louis, MO, the United States). The drugs were dissolved in distilled water and stored at -20°C.

Statistical analysis: Results were expressed as Mean±SEM The comparison between control and the treated samples was analyzed using Student's t-test and $p<0.05$ was considered to be significant.

RESULTS

The methanolic extract of GA showed a myorelaxant effect on the spontaneous contractions and a spasmolytic effect since it reduced induced contraction by spasmogenes agents (Ach) 10^{-5} M and high KCl (100 mM).

Myorelaxant effect of GA: The effect of pretreatment with GA (0.8; 1.6; 2.4; 3.2 and 6.4 mg mL^{-1}) was investigated on spontaneous contractions of rabbit jejunum. The amplitude and the tone of spontaneous contractions under GA were reduced in a concentration-dependent manner.

The fall of the amplitude is not significant before 2.4 mg mL^{-1} of GA. Indeed, the concentrations of 2.4, 3.2

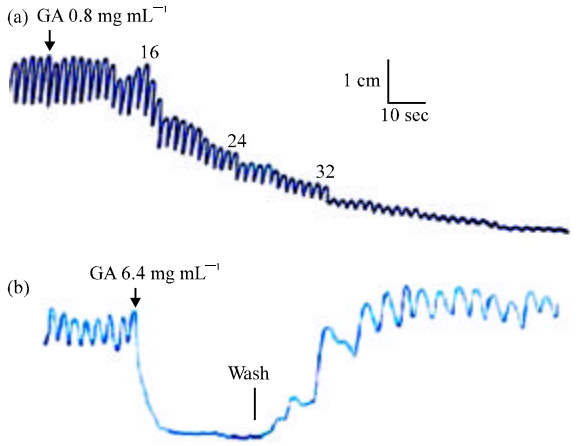

Fig. 1: Typical tracing of rabbit jejunum preparation showing the amplitude, the rate and the basal tone of contractile activity before and after GA. (a) GA (0.8, 1.6, 2.4 and 3.2 mg mL^{-1}). (b) GA (6.4 mg mL^{-1}). Note that contractile activity was restored after washing

and 6.4 mg mL^{-1} decreased significantly the amplitude respectively by 55.62±9.36% ($p<0.05$); 77.11±11.87% ($p<0.01$) and by 88.29±4.07 ($p<0.001$). At 3.2 and 6.4 mg mL^{-1} of GA, the rate of the spontaneous contractions was reduced significantly by 36.25±13.44% ($p<0.001$) and 39.68±12.77% ($p<0.001$), respectively. In the Fig. 1a, a typical tracing shows the myorelaxant effect of GA extract which was completely reversed after washing the preparation, suggesting a non toxic effect of GA at the concentration of 6.4 mg mL^{-1} (Fig. 1b).

Cumulative concentrations of GA extract (1.6, 2.4, 3.2 and 6.4 mg mL^{-1}) decreased significantly the basal tone of the jejunum respectively to -238.64±43.94% ($p<0.05$); -329.55±83.76% ($p<0.05$); -575.76±139.57% ($p<0.01$) and -465±64.56% ($p<0.01$). The fall induced by adrenalin was taken as 100% (Fig. 2).

To investigate an eventual interference of GA extract with Ca^{2+} influx, the spontaneous contraction of the jejunum was examined in Tyrode Ca^{2+} free in the presence or the absence of the extract. In Tyrode Ca^{2+} free, spontaneous contraction and basal tone were drastically reduced. The addition of Ca^{2+} rapidly restores the jejunal activity, indicating the importance of Ca^{2+} influx in spontaneous contraction (Fig. 3a). As clearly shown in Fig. 3b, the presence of GA extract inhibited the recovery of spontaneous contraction during Ca^{2+} supplementation suggesting that in rabbit jejunum, GA extract exerted a relaxant effect via a restriction of Ca^{2+} influx. This inhibitory effect was completely reversible since

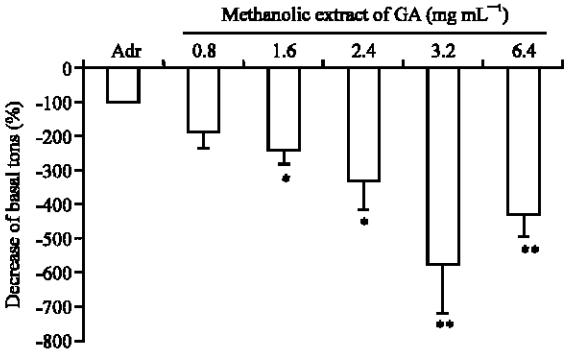

Fig. 2: Effect of methanolic extract of GA on the basal tone of spontaneous activity of isolated rabbit jejunum. The fall in the tonus induced by adrenalin (Adr) was taken as 100% (Mean±SEM, n>6, *$p<0.05$ and **$p<0.01$)

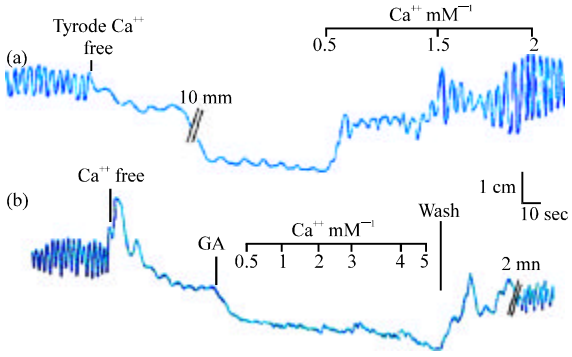

Fig. 3: Typical tracing of contractile activity of rabbit jejunum in Tyrode Ca^{2+} free (a) without and (b) with GA (6.4 mg mL^{-1})

contractile activity was restored a few minutes after washing the preparation with Tyrode.

Spasmolytic effect of GA: Ach (10^{-5} M) caused an increase of the jejunal basal tone which was inhibited by cumulative concentrations of GA as expressed in Fig. 4. Concentrations of GA of 2.4, 3.2 and 6.4 mg mL^{-1} gave significant antispasmodic effects since they decreased the contraction respectively by 83.88±6.90% (**$p<0.01$), 95.5±4.5 % (***$p<0.001$) and 98.8±0.2 (***$p<0.001$).

Typical tracing shows that GA (6.4 mg mL^{-1}) completely reduces the Ach (Fig. 5) and KCl (data not shown) induced contractions of rabbit jejunum.

To elucidate if GA spasmolytic effect is due to Ca^{2+} influx restriction, preparations were exposed for 10 min to Tyrode Ca^{2+} free or to GA (6.4 mg mL^{-1}) and then were challenged with KCl or Ach.

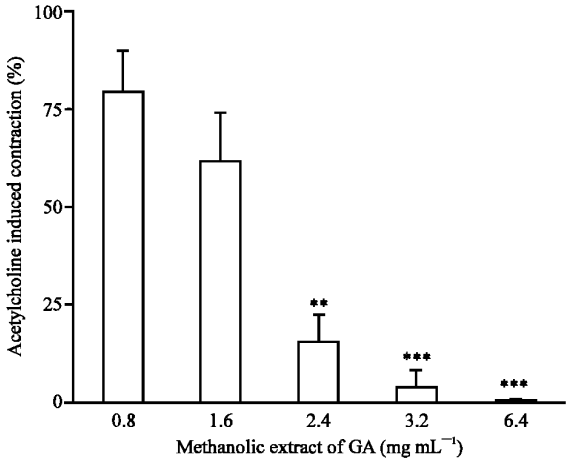

Fig. 4: Effect of methanolic extract of GA on Ach induced contraction on the isolated rabbit jejunum. 100%, was taken as the contraction induced by Ach (10^{-5}M), n>6, **p<0.01, ***p<0.001

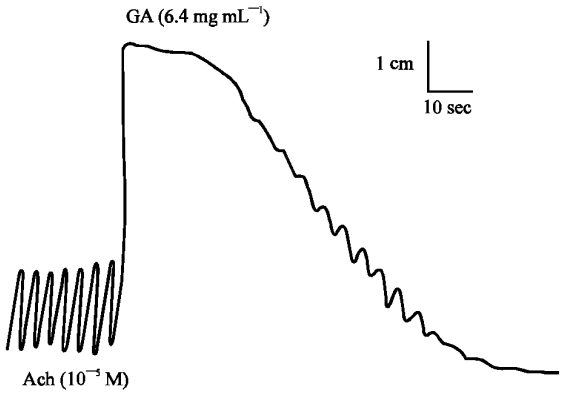

Fig. 5: Typical tracing showing the effect of GA on the induced contraction by Ach on the isolated rabbit jejunum

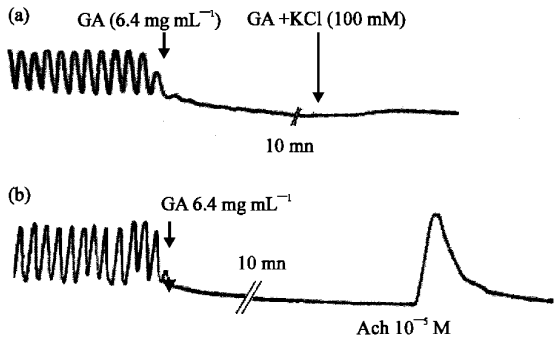

Fig. 6: Typical tracing showing effect of pretreatment (10 min) with GA extract on KCl (a) and Ach (b) induced contraction of rabbit jejunum

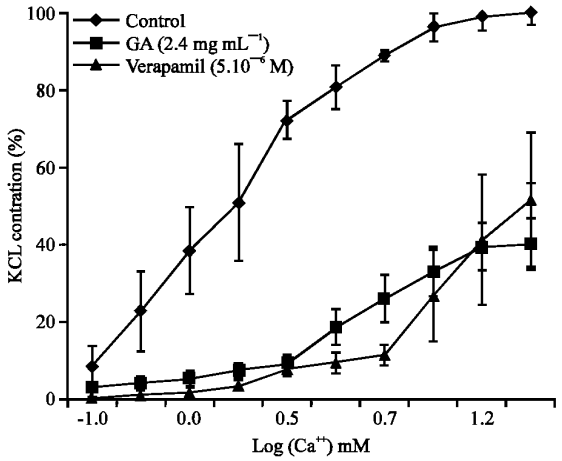

Fig. 7: Effect of exposure to Verapamil (5.10^{-6} M, n = 4) and a sub maximal concentration of GA (2.4 mg mL^{-1}, n = 4) on KCl induced contraction of rabbit jejunum. Note the similar inhibition and the similar right shifting of verapamil and GA

When the jejunum was incubated in Tyrode Ca^{2+} free, KCl induced contraction was almost abolished, tonic Ach induced contraction has disappeared and phasic one was strongly diminished (data not shown). Similar responses were obtained when the jejunum was pre-treated with GA as showed in Fig. 6. Pre-treatment with GA extract completely abolished KCl induced contraction suggesting a Ca^{2+} channel blockade as GA spasmolytic effect (Fig. 6a), while it abolished tonic phase of Ach induced contraction and drastically diminished phasic one (Fig. 6b).

In order to investigate if the spasmolytic effect of GA extract is due to the blockade of the voltage-dependent Ca^{2+} channels, we conducted experiments on tissues incubated in high KCl (100 mM) Ca^{2+} free in presence of verapamil (5.10^{-6} M) or GA (2.4 mg mL^{-1}). As shown in Fig. 7, verapamil inhibited the high K^+ contracted tissues. Furthermore, a sub-maximal concentration of GA (2.4 mg mL^{-1}) shifted the Ca^{2+} dose response curve to the right mimicking the effect of verapamil and suggesting that the spasmolytic effect of GA is mediated probably via a restriction of Ca^{2+} influx through blockade of voltage-dependent Ca^{2+} channel.

DISCUSSION

Our study shows that methanolic extract of GA has an evident relaxant and a spasmolytic effect on rabbit jejunum since it decreases a tone of its spontaneous contraction and reduces Ach and high potassium (KCl 100 mM) induced contraction of it.

It is well established that the spontaneous contractions of the jejunum are initiated by an influx of Ca^{2+} via voltage operated Ca^{2+} channel (Bolton, 1979; Karaki *et al*., 1997). This influx is important to maintain the basal tone of smooth muscle (Sanders, 2001).

Also, smooth muscle relaxation could be obtained by inhibiting Ca^{2+} influx (Beleslin *et al*., 1985; Godfraind *et al*., 1986; Murillo *et al*., 1994). Therefore, inhibition of spontaneous contractions and decrease of the basal tone of rabbit jejunum in the presence of GA could be related to Ca^{2+}channel blockade. To investigate whether GA extract interacts with Ca^{2+} influx, jejunum was exposed to a Ca^{2+} free Tyrode solution. Present results show that GA extract inhibited the restored spontaneous contraction obtained when Ca^{2+} was added to the bath, suggesting that the plant extract probably acts at least by a Ca^{2+} channel blockade since spontaneous contraction of smooth muscle mainly involves those channels (Karaki *et al*., 1997; Boddy and Daniel, 2004; Grasa *et al*., 2004). Present results are in accord with many others reported (Fatehi *et al*., 2004; Gilani *et al*., 2005; Oliveira *et al*., 2006; Ghayur and Gilani, 2006; Estrada-Soto *et al*., 2007; Moazedi *et al*., 2007; Perez-Hernandez *et al*., 2008; Ali *et al*., 2009; Brankovic *et al*., 2009; Gilani *et al*., 2010) who demonstrated that spasmolytic effect of the plant extract was mainly mediated through Ca^{2+} antagonism.

It is well known that high K^+ induced sustained contraction is mainly due to an increase of Ca^{2+} influx via the voltage operated channel (Fleischmann *et al*., 1994; Pérez-Guerrero *et al*., 1997; Grasa *et al*., 2004; Gilani *et al*., 2005). Indeed, high K^+ is ineffective in the absence of external Ca^{2+} (Abe *et al*., 1996). In the other hand, Ach induces smooth muscle contraction via inositol phosphates (IP_3) pathway, which mediated Ca^{2+} release from the sarcoplasmic reticulum (Branding and Sneddon, 1980; Bolton and Imaizumi, 1996; Bolton *et al*., 2004; Gordienko *et al*., 2008). According to Gordienko *et al*. (2008), IP_3 Ca^{2+} release is even facilitated by Ca^{2+} influx through voltage operated channels. Thus, it is possible to speculate that the GA extract might cause the spasmolytic effect through the inhibition of extracellular influx. Therefore, blockade of voltage operated Ca^{2+} channel could inhibit Ach and high KCl induced contraction.

In this study, pre-treatment of jejunum with GA extract inhibits Ach and completely abolished KCl induced contraction. These results suggest that GA spasmolytic effect is probably due at least to Ca^{2+} voltage channel blockade since KCl-induced jejunum contractions and those of Ach are mainly due to Ca^{2+} influx through voltage-dependent channels (Bolton, 1979; Godfraind *et al*., 1986; Carl *et al*., 1996; Gilani *et al*., 2005; Ghayur and Gilani, 2004).

To confirm that GA effect involves a voltage-dependent Ca^{2+} channels blockade, a Ca^{2+} response curves were realized on jejunum incubated in high K^+ Ca^{2+} free in presence of verapamil a standard spasmolytic agent or a sub maximal concentration of GA. Our results show that verapamil and GA inhibited and right shifted the Ca^{2+} response curves confirming that GA extract acts through a voltage-dependent Ca^{2+} channels blockade.

The results of Bello *et al*. (2002) are quite different from ours, since GA extracts reduced histamine and serotonin but not Ach induced contraction on rat duodenum. This difference about Ach induced contraction could be explained by the relative contribution of extracellular Ca^{2+} in smooth muscle contraction which depends on the animal and on the intestinal segment studied, namely rabbit instead of rat and jejunum instead of duodenum (Karaki and Weiss, 1988; Sanders, 2001; Murillo *et al*., 1994).

CONCLUSION

Present results show that GA has relaxant and spasmolytic effect on rabbit jejunum which probably involves a decrease in Ca^{2+} influx at least by a voltage Ca^{2+} channel blockade. These results also confirm the traditional medicinal use in Morocco of GA for the treatment of gastrointestinal disorders.

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REFERENCES

Abe, F., H. Karaki and M. Endoh, 1996. Effects of cyclopiazonic acid and ryanodine on cytosolic calcium and contraction in vascular smooth muscle. Br. J. Pharmacol., 118: 1711-1716.

Ali, N., A. Bashir, B. Shumaila, S. Jehandar, A. Sadiq and A. Manzoor, 2009. Calcium channel blocking activities of *Withania coagulans*. Afr. J. Pharm. Pharmacol., 3: 439-442.

Bashir, S., K.H. Janbaz, Q. Jabeen and A.H. Gilani, 2006. Studies on spasmogenic and spasmolytic activities of *Calendula officinalis* flowers. Phytother. Res., 20: 906-910.

Beech, D.J., I. Mackenzie, T.B. Bolton and M.O. Christen, 1990. Effects of pinaverium on voltage-activated calcium channel currents of single smooth muscle cells isolated from the longitudinal muscle of the rabbit jejunum. Br. J. Pharmacol., 99: 374-378.

Beleslin, D.B., R. Samardzic, B. Terzic and D. Jovanovic-Micic, 1985. The effect of nifedipine and verapamyl on the pendular movements of rabbit isolated ileum. J. Pharmacol. Physiol., 37: 276-277.

Bello, R., L. Moreno, E. Primo-Yufera and J. Esplugues, 2002. *Globularia alypum* L. extracts reduced histamine in and serotonin contraction *in vitro*. Phytother. Res., 164: 389-392.

Ben Sassi, A., F. Harzallah-Skhiri, W. Borgi, N. Chouchène and M. Aoumin, 2007. Effects of the methanolic extract of *Chrysanthemum trifurcatum* (Desf.) Batt and Trab on rat duodenal motility. C. R. Biol., 330: 226-230.

Boddy, G. and E.E. Daniel, 2004. Role of L-Ca(2+) channels in intestinal pacing in wild-type and W/W (V) mice. Am. J. Physiol. Gastrointest. Liver Physiol., 288: 439-446.

Bolton, T.B., 1979. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev., 59: 606-718.

Bolton, T.B. and Y. Imaizumi, 1996. Spontaneous transient outward currents in smooth muscle cells. Cell Calcium, 20: 141-152.

Bolton, T.B., D.V. Gordienko, O.V. Povstyan, M.I. Harhun and V. Pucovsky, 2004. Smooth muscle cells and interstitial cells of blood vessels. Cell Calcium, 35: 643-657.

Branding, A.F. and P. Sneddon, 1980. Evidence for multiple sources of calcium for activation of the contractile mechanism of guinea-pig taenia coli on stimulation with carbachol. Br. J. Pharmacol., 70: 229-240.

Brankovic, S.V., D.V. Kitic, M.M. Radenkovic, S.M. Veljkovic and T.D. Golubovic, 2009. Calcium blocking activity as a mechanism of the spasmolytic effect of the essential oil of Calamintha glandulosa Silic on the isolated rat ileum. Gen. Physiol. Biophys., 28: 174-178.

Caldes, G., B. Prescott and J.R. King, 1975. A potential antileukemic substance present in *Globularia alypum*. Planta Med., 27: 72-76.

Carl, A., H.K. Lee and K.M. Sanders, 1996. Regulation of ion channels in smooth muscles by calcium. Am. J. Physiol., 271: C9-34.

Cortes, A.K., A.J. Delgadillo, M. Hurtado, A.M. Dominguez-Ramirez, J.R. Medina and K. Aoki, 2006. The antispasmodic activity of *Buddleja scordioides* and *Buddleja perfoliata* on isolated intestinal preparations. Biol. Pharm. Bull., 29: 1186-1190.

Dar, A. and S. Channa, 1999. Calcium antagonistic activity of *Bacopa monniera* on vascular and intestinal smooth muscles of rabbit and guinea-pig. J. Ethnopharmacol., 66: 167-174.

Delaey, C., K. Boussery, J. Breyne, B. Vanheel and J. van de Voorde, 2007. The endothelium-derived hyperpolarising factor (EDHF) in isolated bovine choroidal arteries. Exp. Eye Res., 84: 1067-1073.

Eddouks, M., M. Maghrani, A. Lemhadri, M.L. Ouahidi and H. Jouad, 2002. Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalt). J. Ethnopharmacol., 82: 97-103.

Essafi, N., S. Khlifi, L. Kerhoas, A. Kolmann, A. El-Abbouyi and P.H. Ducrot, 2005. Antioxidant constituents of the aerial parts of *Globularia alypum* growing in Morocco. J. Nat. Prod., 68: 1293-1296.

Essafi, N., S. Khlifi, A. Kolmann, L. Kerhoas, A. El-Abbouyi and P.H. Ducrot, 2006. Iridoid Glucosides from the aerial parts of *Globularia alypum* L. (Globulariaceae). Chem. Pharm. Bull., 54: 85-88.

Estrada-Soto S., A. Rodríguez-Avilez, C. Castaneda-Avila, P. Castillo-Espana, G. Navarrete-Vázquez, L. Hernandez and F. Aguirre-Crespo, 2007. Spasmolytic action of *Lepechinia caulescens* is through calcium channel blockade and NO release. J. Ethnopharmacol., 114: 364-370.

Eun, A.K., W.S. Park, Y.K. Son, J.H. Ko and I.D. Jung *et al*., 2010. Calcium channel inhibitor, verapamil, inhibits the voltage-dependent K^+ channels in rabbit coronary smooth muscle cells. Biol. Pharm. Bull., 33: 47-52.

Fatehi M., F. Farifteh and Z. Fatehi-Hassanabad, 2004. Antispasmodic and hypotensive effects of *Ferula asafoetida* gum extract. J. Ethnopharmacol., 91: 321-324.

Fleischmann, B.K., R.K. Murray and M.I. Kotolikoff, 1994. A voltage window for sustained elevation of cytosolic calcium in smooth muscle cells. Proc. Natl. Acad. Sci. USA., 91: 11914-11918.

Ghayur, M.N. and A.H. Gilani, 2004. Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. Dig. Dis. Sci., 50: 1889-1897.

Ghayur, M.N. and A.H. Gilani., 2006. tracheal relaxant effects of *Sarcococca saligna*. Arch. Pharm. Res., 29: 990-997.

Gilani, A.H., Q. Jabeen, M.N. Ghayur, K.H. Janbaz and M.S. Akhtar, 2005. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. J. Ethnopharmacol., 98: 127-135.

Gilani, A.H., S.U.R. Mandukhail, J. Iqbal, M. Yasinzai, N. Aziz, A. Khan and N.U. Rehman, 2010. Antispasmodic and vasodilator activities of *Morinda citrifolia* root extract are mediated through blockade of voltage dependent calcium channels. BMC Complement Altern Med., 10: 2-2.

Godfraind, T., R. Miller and M. Wibo, 1986. Calcium antagonist and calcium entry blockade. Pharmacol. Rev., 38: 321-416.

Gordienko, D.V., M.I. Harhun, M.V. Kustov, V. Pucovsky and T.B. Bolton, 2008. Sub-plasmalemmal [Ca^{2+}]i upstroke in myocytes of the guinea-pig small intestine evoked by muscarinic stimulation: IP3R-mediated Ca^{2+} release induced by voltage-gated Ca^{2+} entry. Cell Calcium, 43: 122-141.

Goto, K., Y. Kasuya, N. Matsuki, Y. Takuwa and H. Kurihara *et al*., 1989. Endothelin activates the dihydropyridine-sensitive, voltage-dependent Ca^{2+} channel in vascular smooth muscle. Proc. Natl. Acad. Sci., 86: 3915-3918.

Grasa, L., E. Rebollar, M.P. Arrubeo, M.A. Plaza and M.D. Murillo, 2004. The role of Ca^{2+} in the contractility of rabbit small intestine *in vitro*. J. Physiol. Pharmacol., 55: 639-650.

Horowitz, A., C.B. Menice, R. Laporte and K.G.Morgan, 1996. Mechanisms of smooth muscle contraction. Physiol. Rev., 76: 967-1003.

Itoh, T., Y. Kanmura, H. Kuriyama and H. Suzuki, 1984. Nisoldipine-induced relaxation in intact and skinned smooth muscles of rabbit coronary arteries. Br. J. Pharmacol., 83: 243-258.

Jothimanivannan, C., R.S. Kumar and N. Subramanian, 2010. Anti-inflammatory and analgesic activities of ethanol extract of aerial parts of *Justicia gendarussa* Burm. Int. J. Pharmacol., 6: 278-283.

Jouad, H., M. Maghrani and M. Eddouks, 2002. Hypoglycaemic effect of *Rubus fructicosis* L. and *Globularia alypum* L. in normal and streptozotocin-induced diabetic rats. J. Ethnopharmacol., 81: 351-356.

Karaki, H. and G. Weiss, 1988. Mini-review: Calcium release in smooth muscles. Life Sci., 42: 111-122.

Karaki, H., H. Ozaki, M. Hori, M. Mitsui-Saito and K. Amano *et al*., 1997. Calcium movements, distribution and fuctions in smooth muscle. Pharmacol. Rev., 49: 157-230.

Kocyigit, I., A. Unal, M. Sipahioglu, B. Tokgoz, O. Oymak and C. Utas, 2010. Peritoneal dialysis-related peritonitis due to *Neisseria weaveri*: The first case report. Int. J. Pharmacol., 30: 116-117.

Merzouki, A., F. Ed-Derfoufi and J.M. Mesa, 2000. Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). Fitoterapia., 71: 278-307.

Moazedi, A.A., D.N. Mirzaie, S.M. Seyyednejad, M.R. Zadkarami and A. Amirzargar, 2007. Spasmolytic effect of *Petroselinum crispum* (Parsley) on rat's ileum at different calcium chloride concentrations. Pak. J. Biol Sci., 10: 4036-4042.

Murillo, M.D., M.A. Plaza, M.J. de Pedro and M.P. Aruuebo, 1994. The effect of Ca^{2+} antagonists on spontaneous motility from sheep duodenum. J. Pharmacol. Physiol., 46: 138-140.

Ohya, Y., K. Terada, K. Kitamura and H. Kuriyama, 1987. D600 blocks the Ca^{2+} channel from the outer surface of smooth muscle cell membrane of the rabbit intestine and portal vein. Pflugers Arch., 408: 80-82.

Oliveira, R.C., J.T. Lima, L.A. Ribeiro, J.L. Silva and F.S. Monteiro *et al*., 2006. Spasmolytic action of the methanol extract and isojuripidine from *Solanum asterophorum* Mart. (*Solanaceae*) leaves in guinea-pig ileum. Z. Naturforsch C., 61: 799-805.

Parekh, A.B. and A.F. Brading, 1991. The sources of calcium for carbachol-induced contraction in the circular smooth muscle of guinea-pig stomach. Br. J. Pharmacol., 104: 412-418.

Perez-Guerrero, C., J. Suarez, M.D. Herrera and E. Marhuenda, 1997. Spasmolytic effect of tetrazepam on rat duodenum and guinea pig ileum. Pharmacol. Res., 35: 493-497.

Perez-Hernandez, N., H. Ponce-Monter, J.A. Medina and P. Joseph-Nathan, 2008. Spasmolytic effect of constituents from *Lepechinia caulescens* on rat uterus. J. Ethnopharmacol., 115: 30-35.

Radhika, T., P. Mahendar, A. Venkatesham, A.R.N. Reddy, Y.N. Reddy, A. Sadanandam and T. Christopher, 2010. Hypoglycemic activity of red kino tree in normal and streptozotocin induced diabetic rats. Int. J. Pharmacol., 6: 301-305.

Sanders, K.M., 2001. Signal transduction in smooth muscle: Mechanism of calcium handling in smooth muscles. J. Applied Physiol., 91: 1438-1449.

Shimizu, S., H. Yokoshiki, H.N. Sperelakis and R.J. Paul, 2000. Role of voltage-dependent and Ca($^{2+}$)-activated K(+) channels on the regulation of isometric force in porcine coronary artery. J. Vasc. Res., 37: 16-25.

Skim, F., A. Kaaya, J.T. Jaouari and H.B. Lazrek, 1999. Pharmacological studies of two antidiabetic plants: *Globularia alypum* and *Zygophyllum gaetulum*. Therapy, 54: 711-715.

Terada, K., Y. Ohya, K. Kitamura and H. Kuriyama, 1987. Actions of flunarizine, a Ca^{++} antagonist, on ionic currents in fragmented smooth muscle cells of the rabbit small intestine. J. Pharmacol. Exp. Ther., 240: 978-983.

Thaina, P., P. Tungchareon, M. Wongnawa, W. Reanmongkol and S. Subhadhiraskul, 2008. Uterine relaxant effects of *Curcuma aeruginosa* Roxb. Rhizome extracts. J. Ethnopharmacol., 121: 433-443.

Zennaki, S., D. Krouf, D. Taleb-Senouci and M. Bouchenak, 2009. *Globularia alypum* L. lyophilized methanolic extract decreases hyperglycemia and improves antioxidant status in various tissues of streptozotocin-induced diabetic rats. J. Complementary Integrative Med., 6: 1-4.

Zhou, S., L. Liu, X. Yang, S. Wu and G. Chen, 2010. Paraoxon attenuates vascular smooth muscle contraction through inhibiting Ca^{2+} influx in the rabbit thoracic aorta. J. Biomed. Biotechnol., 2010: 1-9.

Ziyyat, A., A. Legssyer, H. Mekhfi, A. Dassouli, M. Serhrouchni and W. Benjelloun, 1997. Phytotherapy of hypertension and diabetes in oriental Morocco. J. Ethnopharmacol., 58: 45-54.