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

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**Abstract:** The aim of this study was to investigate the myorelaxant and the spasmodic 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 spasmodic 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 (Radha et al., 2010; Jothimaniyavaman et al., 2010; Kocyigit et al., 2010) and especially in gastrointestinal transit disorders (Thaino 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 blue to purple. In Morocco, it locally called Ain Irmel and its leaves are used in traditional medicine as a hypoglycemic, a laxative, a purgative, a myorelaxant and an antispasmodic remedy (Ziyat 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, Zennani 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

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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 Ca2+ channels to cause an influx of Ca2+ and finally induce muscle contraction (Godfraind et al., 1986; Horowitz et al., 1996; Ghyur and Gilani, 2004; Gilani et al., 2005; Thaina et al., 2008).

Also, it is well known that Ca2+ channel blockers as verapamil inhibit the Ca2+ 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 Ca2+ restriction influx has been often proposed as the origin of myorelaxant and spasmylytic effects of many medicinal plants (Fatehi et al., 2004; Estrada-Soto et al., 2007; Moazed et al., 2007; Brankovic et al., 2009; Gilani et al., 2010), the present study was designed to see whether the spasmylytic activity of GA extract on isolated rabbit jejunal is due to possible Ca2+ channel blockade. Also, the present paper was conducted to validate GA popular use as spasmylytic 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 jejunal 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 CaCl2, 2H2O, 0.5 MgCl2, 6H2O, 11.9 NaHCO3, 0.42 NaH2PO4 and 5.56 Glucose (bubbled continuously with 95% O2 and 5% CO2, 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.

Spasmylytic effect was studied on tone increase induced by Ach 10-4 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 Ca2+ channel blockade, the jejunum was replaced in Ca2+ free Tyrode solution with EDTA (2 mM) leading to the removal of Ca2+ from tissues. After the abolition of the spontaneous contraction, Ca2+ 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 Ca2+ free Tyrode’s solution with EDTA (2 mM) and then to the high KCl (100 mM) Ca2+ free solution during 10 min. Controlled Ca2+ response curve was obtained with cumulative addition of CaCl2 (0.1-20 mM). The same experiences were repeated in presence of sub-maximal concentration of GA (2.4 mg mL-1) or verapamil (5.10-4 M), a standard voltage dependent Ca2+ channel blocker (Ohyay et al., 1987; Terada et al., 1987).

**Drugs:** Ach, adrenaline 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 spasmylytic 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
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±3.44% (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 -28.64±14.39% (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

**Spasmyloytic 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), 95.54±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 spasmyloytic 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 (10⁻⁷M), n=6, **p<0.01, ***p<0.001

When the jejunum was incubated in Tyrode Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ channels, we conducted experiments on tissues incubated in high KCl (100 mM) Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ influx through blockade of voltage-dependent Ca²⁺ 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; Moazed et al., 2007; Perez-Hernandez et al., 2008; Ali et al., 2009; Brankovic et al., 2009; Gilani et al., 2010) who demonstrated that spasmyltic 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; Perez-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 Imaiizumi, 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 spasmyltic 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 spasmyltic 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 spasmyltic 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 spasmyltic 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


