Antispasmodic Effect of Aqueous Extract of *Rubia tinctorum* L. Roots on Rodents

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**ARTICLE INFORMATION**

**ABSTRACT**

*Rubia tinctorum* L. (Rubiaceae) root is traditionally used in Morocco for treating the gastro-intestinal diseases. The aim of the present study was to assess the antispasmodic effects of the aqueous extract of this root plant (AR) on rodents. The effect of the AR on spontaneous contraction was studied, *in vitro*, on jejunum isolated from rabbits in the presence and absence of adrenergic inhibitors (Prasosin, Propranolol, and Yohimbine). This effect was assessed on jejunum of rats pre-contracted by carbachol and a KCl rich KHB. Also cumulative concentration-response curves were obtained for Cch (10⁻⁸ - 10⁻⁶ M) and CaCl₂ (0.1-10 mM), in the absence or presence of the AR. The AR caused a dose dependent reversible inhibition (0.1-5 mg/ml) on basic rabbit jejunum contractions, with a total relaxation at 5 mg/ml. Pretreatment with adrenergic inhibitors (Prasosin, Propranolol, and Yohimbine) did not exhibit any action on the effect of this extract. Also extract inhibited carbachol (CCh) and KCl (75 mM) induced contractions in rat jejunum at a dose-dependent manner with IC₅₀ value of 1.60 ± 0.14 ng/ml and 1.06 ± 0.21 mg/ml respectively. This extract has exhibited an inhibitory effect on the dose-response curves induced by CCh and CaCl₂ on rat jejunum and significantly reduced the maximal response in a concentration-dependent manner, similar to the effect of non competitive antagonist of muscarinic receptors and calcium channels respectively. The results showed an antispasmodic effect which might explain the traditional use of Rubia tinctorum L. in gastrointestinal disorders.

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1. Introduction

*Rubia tinctorum* L. (Rubiaceae) root have been used in antidiarrheic, anaemia, diuretic, antilithiasic, aphrodisiac and as natural dye for textile in Morocco (Bellakhdar, 1997). The plant is reported to possess antimicrobial (Kalyoncu et al., 2006), antifungal activities (Manojlovic et al., 2005). It produces various kinds of anthraquinone pigments in its roots, one of them being the most abundant is alizarin which has been used for dyeing textile (Angelini et al., 1997), purpurin, xanthopurpurin (Knorr et al., 1993), and lucidin that was demonstrated to be most potent mutagenic in bacterial and mammalian systems (Kawasaki et al.,
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1992). In continuation of our previous study on the anti-diarrhoeal activity of the crude aqueous extract of Rubia tinctorum L. roots (AR) in rodents (Karim et al., 2010), we aimed to investigate the effects of this extract on the isolated smooth muscle of rat and rabbit jejunum to evaluate their popular use as spasmolytic and their possible mechanism of action.

2. Materials and Methods

2.1 Plant material

The roots of Rubia tinctorum L. were collected locally from north eastern area of Morocco, and identified by Professor Benyounes Haoului. A voucher specimen (N° 50759) was previously deposited in Scientific Institute of Rabat. The roots of this plant were air-dried, boiled in distilled water. The water was removed under vacuum in a rotary evaporator until dryness. The percentage yields based on the dried starting material was 12.9% for dried aqueous extract.

2.2 Solutions and Drugs

1. The solutions used had the following composition (mM):
   - Normal Krebs-Henseleit Bicarbonate (KHB): (mM) NaCl, 118; KCl, 4.7; CaCl$_2$, 2.5; MgSO$_4$, 1.2; NaHCO$_3$, 25; KH$_2$PO$_4$, 1.2 and glucose 10.
   - High K$^+$ KHB (75mM): NaCl, 48; KCl, 75; CaCl$_2$, 2.5; MgSO$_4$, 1.2; NaHCO$_3$, 25; KH$_2$PO$_4$, 1.2 and glucose 10.
   - Calcium-free high K$^+$ KHB (75mM); NaCl, 48; KCl, 75; CaCl$_2$, 0.0; MgSO$_4$, 1.2; NaHCO$_3$, 25; KH$_2$PO$_4$, 1.2 and glucose 10.
   - Calcium-free KHB; NaCl, 121.7; KCl, 4.7; CaCl$_2$, 0.0; MgSO$_4$, 1.2; NaHCO$_3$, 25; KH$_2$PO$_4$, 1.2; glucose 10 and EDTA, 0.1, made up in distilled water, the pH was adjusted to 7.4.

2. The following drugs were used for the experiments: Carbamylcholine chloride (Carbachol, CCh) was purchased from Prolabo, and propranolol hydrochloride, prazosin hydrochloride, and yohimbine hydrochloride from Sigma.

2.3 Spasmolytic study

Wistar rats (200–250 g) and New Zealand rabbit (1.5–2 kg) either sex were used for this study. The animals were housed at the animal House of the Department of Biology, Mohammed the First University, Oujda, Morocco. A commercial diet and tap water were provided ad libitum. Animals were fasted for 24 h before the studies with free access to water. All procedures concerning animals were carried out in an ethically proper way by following guidelines as set by the World Health Organization and conform to the European Community guiding principles in the care and use of animals (86/609/CEE, CE Off J No. L358, 18 December 1986).

The spasmolytic activity of the plant materials was studied using isolated Wistar rat and rabbit jejunum preparations. A portion of jejunum (2 cm) was removed and mounted in 10 ml organ baths containing Krebs-Henseleit Bicarbonate solution (KHB). The bath solution was maintained at 37°C, pH 7.4 and gassed continuously with air bubbling. A 60 min equilibration period was allowed during which the physiological solution was changed every 15 min. Extracts were added directly to the organ bath. Each concentration of the extract was at least 7 min in contact with the tissue before its effect was evaluated.

2.3.1 Spontaneous contractions and effect of extract on adrenergic receptors of rabbit jejunum

After stabilization of smooth muscle spontaneous contractions of rabbit jejunum, the cumulative doses of aqueous extract were added (0.1-5 mg/ml). So as to assess the aqueous extract effect on adrenergic receptors, the adrenergic inhibitors were added before at the same time (Prasosin (5.10$^{-5}$ M), Propranolol (5.10$^{-5}$ M), and Yohimbine (5.10$^{-5}$ M),,)

2.3.2 Relaxant effect on K$^+$ or CCh induced contractions

The jejunum was contracted with K$^+$ (75 mM) (Farre et al., 1991; Tortoriello & Aguilar-Santamaria, 1996), or Carbamylcholine chloride (Carbachol, CCh, 10$^{-6}$ M) to a maintained tone, at this point the AR was added to the bath.

2.3.3 Inhibition of dose-response curves to Carbachol

Cumulative dose-response curves for Carbachol (CCh) were obtained for the tissues (Sánchez et al., 1995). After a stabilization period of 60 min, CCh (10$^{-6}$ - 10$^{-4}$ M) was added to the organ bath, and different doses of the AR extract were added to the bath 5 min before constructing the dose-response curve of the agonist.

2.3.4 Inhibition of dose-response curves to CaCl$_2$

After an initial incubation period of 60 min in normal KHB’s solution, the nutrient solution was replaced by calcium-free KHB during 15 min, and then replaced by calcium-free hyperpotasssic medium (75 mM). Cumulative dose-response curves to CaCl$_2$ (0.1, 0.3, 1, 3, 10 mM) were obtained in the presence of different doses of aqueous extract (Farre et al., 1991).
2.4 Statistics

The results are expressed as means ± S.E.M. The statistical significance of data was analyzed using Student’s t-test for comparing the control and the various groups, using Primer of biostatistics version 4.02 (windows McGraw Hill, Stanton A. Glantz), P<0.05 was considered as significant.

3. Results

When tested on hyperactive smooth muscle preparations, spontaneously contracting rabbit jejunums, the AR exhibited a relaxant effect in a dose-dependent (0.1-10 mg/ml) manner, and showed a total persistent inhibition at 5 mg/ml of rabbit jejunal muscle until the rinsing (Figure 1). This effect is reversible.

The aqueous extract produced its relaxant effect in the presence of the α1, α2 and β adrenergic receptors inhibitors Prasosin, Propranolol and Yohimbine respectively (Figure 2).

The extract of this plant in a concentration-dependent manner inhibited the rat jejunal contraction induced by CCh (10⁻⁶ M), an analogue of acetylcholine with IC₅₀ value of 1.60 ± 0.14 mg/ml (Figure 3). The extract had a significant inhibitory effect on CCh concentration–response curve, reducing the maximum induced contraction. It shifted the CCh-response curves to the right and down (Figure 4).

Moreover, to assess whether the spasmolytic effect was mediated through adrenergic receptors (Amos et al., 1998).

4. Discussion

The present data show that the aqueous extracts of Rubia tinctorum L. roots exert concentration dependent reversible inhibitory effects on contractile responses in smooth muscle of isolated rabbit jejunums. When tested in the presence of Prasosin, Propranolol and Yohimbine (at the same time), α1, α2 and β adrenergic receptor blockers respectively, inhibitory effect of the aqueous extract remained unchanged, suggesting that the effect were not mediated through adrenergic receptors (Amos et al., 1998).

Figure 1. Effect of aqueous extract of Rubia tinctorum L. roots on the isolated rabbit’s jejunum

Figure 2. Effect of aqueous extract of Rubia tinctorum L. roots in presence of different antagonists on contractions of isolated rabbit jejunum. (Prasosin (5.10⁻⁵ M), Propranolol (5.10⁻⁵ M), Yohimbine (5.10⁻⁵ M)). A) Effect of aqueous extract only. B) Effect of the pretreatment with propranolol, yohimbine and prazosin on the inhibitory effect of the extract on rabbit jejunum.

To see whether the spasmolytic effect was mediated through the blockade of Ca²⁺ influx, a high dose of K⁺ (75 mM) was introduced to depolarize the tissue. The contractions induced by high K⁺ are dependent on the entry of Ca²⁺ into the cells through voltage-dependent channels (Bolton, 1979) and a substance which can inhibit K⁺-induced contractions is therefore, considered to be a calcium channel blocking (Godfraind et al., 1986). Thus, inhibition of
high K⁺ (75 mM)-induced contraction of rat jejunum by aqueous extract of this plant may reflect the restricted Ca²⁺ entry via voltage-dependent channels. This hypothesis was further strengthened when pretreatment of the tissue with the plant extract caused a concentration-dependent rightward shift in the concentration-response curves of CaCl₂ (Rojas et al., 1996).

**Figure 3.** Relaxant effects of different doses of aqueous extract of Rubia tinctorum L. (mg/ml) on CCh (10⁻⁶ M)-induced contractions. ** P<0.01 and *** P<0.001 were statistically significant difference from control, (±S.E.M, Student’s t-test; n = 6).

The aqueous extract exhibited concentration-dependent relaxation of the jejunum contractions induced by CCh, suggestive of non-specific relaxation (Gilani et al., 2005). The inhibitory effect of this extract on CCh concentration-response is like non-competitive antagonism attenuating the maximum response (Hajhashemi et al., 2000).

Beside the main anthraquinone, alizarin, 36 other anthraquinones have been reported from Rubia tinctorum (Goverdina et al., 2002). These compounds are founded in the plant Senna (Cassia acutifolia pods) that has been used as laxative for centuries (Ben, 1972). Further investigations are necessary to determine which anthraquinones or other products are responsible for this relaxant activity.

**Figure 4.** Cumulative log concentration-response curves (± S.E.M, Student’s t-test; n = 6) for CCh in the presence and absence of Rubia tinctorum L. extract.

Often the use of Rubia by local population is for a short duration. The crude extract was studied for acute oral toxicity using Swiss albino mice and the study shown that the maximum tolerated dose of Rubia extract was 10 mg/ml (Karim et al., 2010). It should better to estimate the concentration of anthraquinone compounds particularly lucidin that was demonstrated to be most potent mutagenic in bacterial and mammalian systems (Kawasaki et al., 1992) and to test their acute effect for its possible genotoxicity.

**Figure 5.** Relaxant effects of different doses of aqueous extract of Rubia tinctorum L. (mg/ml) on KCl (75 mM)-induced contractions. * P<0.05 and *** P<0.001 were statistically significant difference from control, (±S.E.M, Student’s t-test; n = 6).

**Figure 6.** Cumulative log concentration-response curves (± S.E.M, Student’s t-test; n = 6) for CaCl₂ in the presence and absence of aqueous extract of Rubia tinctorum L.
5. Conclusions

As a conclusion, our results support that aqueous extract of *Rubia tinctorum* L. exhibits antispasmodic activity probably by inhibiting calcium influx or perhaps interfere in one of the multiple biochemical processes associated with the influx of calcium into the smooth muscle cells. This study may partly explain the traditional use of the plant in folk medicine to treat certain gastrointestinal disorders. Other investigations are necessary to complete this study.

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