Antispasmodic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileum

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Received: 19 Nov 2006; Revised: 13 Feb 2007; Accepted: 24 March 2007

ABSTRACT

Tecoma stans (L.) Juss or Yellow bells from Bignoniaceae is a ornamental tropical shrub or small tree predominantly found in central, and south America and in Latin America is used traditionally for reducing blood glucose. However, its other pharmacological effects have not been yet elucidated. The aim of present study was to investigate the effect of its leaves extract on rat ileum contractility and involved mechanism(s). *Tecoma stans* Juss hydroalcoholic leaf extract (TLE) was prepared by macerated method using 70% alcohol. Distal segment of ileum (2 cm) from male Wistar rat was mounted in an organ bath containing Tyrode solution (10 ml, pH 7, 37 °C) and pre-contracted by carbachol (CCh, 10 μM) or by KCl (60 mM). The antispasmodic effects of TLE (0.125–2 mg/ml) were studied prior and after 20-30 min incubation of ileum with propranolol (1μM), naloxone (1μM), L-NAME (100 μM), or 5 min incubation with glibenclamide (10 μM) and tetraethylammonium (TEA, 1mM). The effect of TLE on $CaCl_2$-induced contraction in Ca^{2+}-free with high K^+ Tyrode solution was also studied. The CCh- and KCl-induced ileal contractions were reduced by TLE ($P<0.0001$). This effect was not attenuated by propranolol, naloxone, L-NAME, glibenclamide and TEA. In Ca^{2+}-free Tyrode solution with high K^+, cumulative concentrations of $CaCl_2$ induced contractions which were inhibited by TLE dose-dependently. Our results indicate that the *Tecoma stans* (L.) Juss leaf extract induces its antispasmodic effects without involvement β-adrenoceptors, opioid receptors, potassium channels and NO production. It seems that, the calcium channels are involved in this spasmolytic effect.

Keywords: *Tecoma stans* (L.) Juss, Rat, Ileum, Antispasmodic.

INTRODUCTION

Diarrhea continues to be one of the leading causes of mortality and morbidity especially in children in developing countries (1). Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by intestinal hurry, and an excess loss of fluid in the faeces. In some diarrhoeas the secretory component predominates, and other diarrhoeas are characterized by hypermotility (2). Many people nowadays turn to the use of natural product medicine for treatment of intestinal disorders. Natural products have served as a source of medicines for centuries, and about half of the pharmaceuticals in use today are derived from natural products (3). Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care (4). *Tecoma stans* (L.) Juss ex Humboldt, Bonpland & Kunth] from Bignoniaceae family is a semi-evergreen ornamental tropical shrub or small tree originally from Latin American which has been cultivated in Iran (particularly in west and southwest parts) recently. Its leaves are used traditionally in Mexico in order to control diabetes (5, 6). The tecostanine isolated from the leaves is suggested for antihyperglycemic effect of this substance (7). *Tecoma stans* growing in Egypt has two alkaloids called tecomine-1 and tecostanine-2 with hypoglycemic effect in fasting rabbits which are inactive in the absence of pancreas (8, 9). Tecoma is not a toxic because this plant is used in Latin America as a remedy for diabetes and moreover for feeding cattle and goats in Mexico (10). Despite the traditional use of Tecoma, its pharmacological properties on smooth muscle has not been carried out. The aim of the present study, therefore, was to investigate the antispasmodic effect of hydroalcoholic *Tecoma stans* (L.) Juss leaf extract (TLE) on isolated rat ileum and its mechanism(s).

MATERIALS AND METHODS

Chemicals

The salts of Tyrode solution were purchased from Merck (Germany), and carbachol, glibenclamide, tetraethylammonium, propranolol and L-NAME were purchased from Sigma (USA) and naloxone

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from Tolidaru Company (Iran). The extract and all chemicals were dissolved in Tyrode solutions (normal or Ca^{2+}-free) and glibenclamide was first dissolved in DMSO (50 µl) then diluted by Tyrode solution in such a way that the final concentration of DMSO in tissue bath was 0.005 % (W/W) (16). The volume of all chemicals added to the tissue bath did not exceed to more than 5% of organ bath volume.

Plant and extraction

Tecoma stans (L.) Juss leaves were collected (March 2006) at the campus of the Ahwaz Jundishapur University of Medical Sciences and identified by Dr. Khaleghi from Ahwaz Shahid Chamran University, Faculty of Agriculture and Dr. Siahpoosh from Department of Pharmacognosy, Ahwaz Jundishapur University of Medical Sciences (AJUMS). A voucher specimen (No. A07033001P) was deposited at Herbarium of the Faculty of Pharmacy (AJUMS) for further references. To prepare *Tecoma stans* Juss hydroalcoholic leaf extract (TLE) the leaves were dried under shade, graded by an electric blender and powder (20 g) was extracted by macerated method using 70% alcohol (100 ml) for 72 h at room temperature and were shaken several times every day. The mixture was then filtered (Whatman No.1) and the solvent evaporated (yield: 7.72 g). The extract powder was stored at 4º C until being used.

Animals

Male adult Wistar rats (200-250g) were purchased from Animal Facility of Jundishapur Ahwaz University of Medical Sciences and kept at 20-24 ºC and 12h/12h light/dark cycle and free access to food and water. All animals used in this study were treated in accordance with principals and guidelines on Animals Care of Jundishapur Ahwaz University of Medical Sciences. The rats were deprived from food for 24 h before the experiment but had free access to water.

Tissue preparation

Animals were killed by a sharp blow to the head and after the laparatomy, one or two pieces (2 cm) of ileum were dissected from 2 cm above the ileocaecal junction and intraluminal content flushed out with cooled oxygenated Tyrode solution. The tissue was mounted in an organ bath (10 ml) containing Tyrode solution (37 ºC, pH 7.4) between two stainless steel hooks and subjected to continuous air bubbling (11). The lower hook was fixed at the bottom of the organ bath and the upper one was connected to an isotonic transducer (Harvard Transducer). The ileum contractions were recorded (Universal Harvard Oscillograph, UK) under 1 g resting tension, following 60 min for equilibrium period. During this period, the organ bath solution was refreshed every 15 min. The composition of Tyrode solution (mM) was: NaCl (136), KCl (5), $CaCl_2$ (2), $NaHCO_3$ (11.9), NaH_2PO_4 (0.26), $MgCl_2$ (0.98) and glucose (5.6) (11). The ileum contractions were induced by 60 mM of KCl (12) or 10 µM of carbachol (13) and once the plateau of contraction elicited by stimulant was achieved, the extract was added to the organ bath cumulatively (0.125- 2 mg/ml). To study the involvement of some receptors, after recording the spasmolytic effect of the extract (0.125-2 mg/ml) and several washing tissue preparation followed by at least 15 min for rest, the same protocol was carried out in presence of propranolol or naloxone (1 µM, 30 min). L-NAME (100 µM, 20 min) (14) was also used as a nitric oxide synthase inhibitor. The role of the calcium was evaluated by depolarizing the tissue (KCl, 60 mM) in Ca^{2+}-free solution and then calcium chloride was applied to the organ bath cumulatively (0.225-2.7 mM) after ileum incubation (3 min) with different extract concentrations (0.25-2 mg/ml of the extract). Involvement of potassium channels in extract antispasmodic effect on carbachol (CCh, 10 µM)-induced contraction was evaluated by tissue incubation (5 min) with glibenclamide (10 µM) or 1 mM of tetraethylammonium (15). Each antagonist, inhibitor or blocker was applied only on one segment of the ileum.

Statistical analysis

All values in text and figure (as percentage of relaxation or contraction) are expressed as mean±SEM for *n* number animals. Statistical significance of differences between two means was assessed by Student's t test. Multiple means were compared by one-way analysis of variance (ANOVA). *P* values of less than 0.05 were considered to present significant differences.

RESULTS

Effect of extract on KCl- and carbachol- induced ileal contractions

Cumulative concentrations (0.125-2 mg/ml) of TLE attenuated contractions induced by KCl (60 mM, n=9) or CCh (10 µM, n=10) significantly (ANOVA, $P<0.0001$) and in a dose dependent manner as is shown in Figure 1. These two response curves are not significantly different.

Effect of propranolol on extract antispasmodic activity

Incubation of ileum preparation (30 min) with propranolol (1 µM, n=8) as a β-adrenoceptor antagonist did not alter the TLE (0.125-2 mg/ml) spasmolytic effect on ileal contractions induced by KCl (60 mM) as shown in Figure 2.

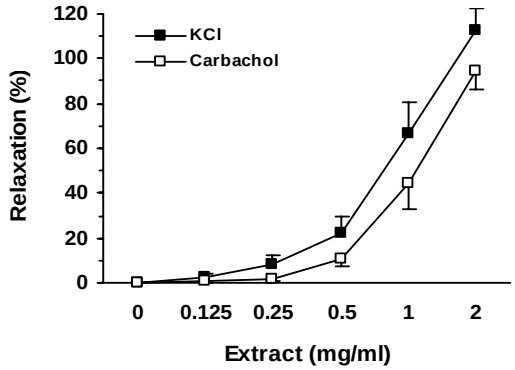

Figure 1. Spasmolytic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileal contractions induced by KCl (60 mM, n=9) or carbachol (10µM, n=14). These two dose-response curves are not significantly different.

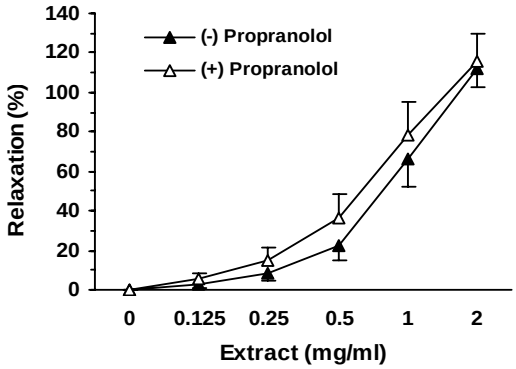

Figure 2. Spasmolytic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileal contractions induced by KCl (60 mM, n=9) before (-) and after (+) tissue incubation (30 min) with propranolol (1 µM, n=8). These two dose-response curves are not significantly different.

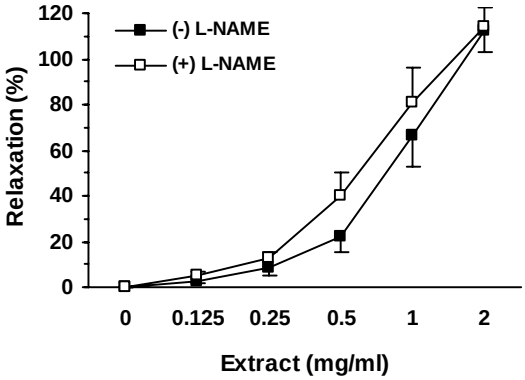

Figure 3. Antispasmodic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileal contractions induced by KCl (60 mM, n=9) before (-) and after (+) tissue incubation (20 min) with L-NAME (100 µM, n=7). Inhibition nitric oxide synthesis has not altered the spasmolytic effect of the extract.

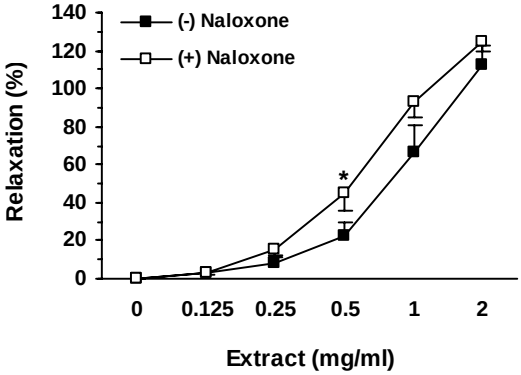

Figure 4. Antispasmodic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileal contractions induced by KCl (60 mM, n=9) before (-) and after (+) tissue incubation (30 min) with naloxone (1 µM, n=8). Naloxone did not reduce the spasmolytic effect of the extract but rather potentiated at 0.5 mg/ml (* P<0.05).

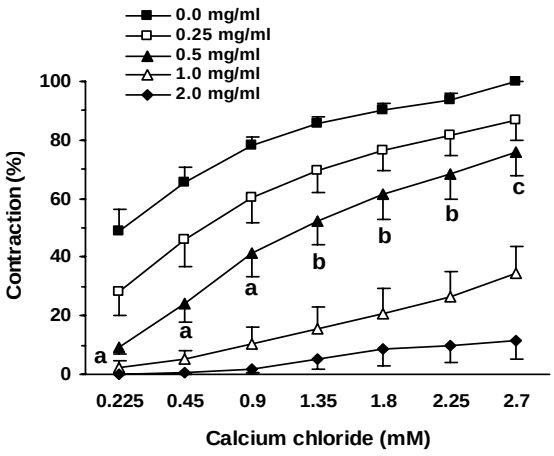

Figure 5. Ileal contractions induced by $CaCl_2$ before (0.0 mg/ml) and after incubation of ileum (3 min) with different concentrations of the *Tecoma stans* (L.) Juss leaf extract. The contraction induced by 2.7 mM of $CaCl_2$ has been considered as 100%. The statistical analysis has been carried out only between 0.0 mg/ml and 0.5 mg/ml of the extract (a: P<0.001, b: P<0.01, c: P<0.05).

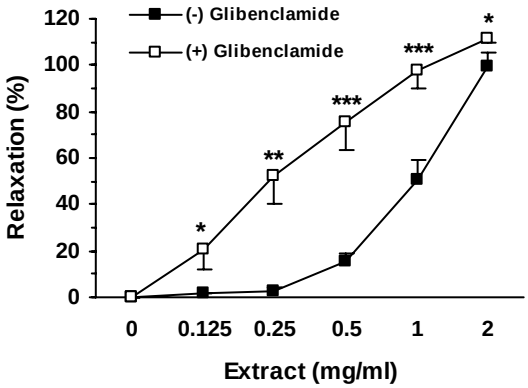

Figure 6. Spasmolytic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileal contractions induced by CCh (10 µM, n=14) before (-) and after (+) tissue incubation (5 min) with glibenclamide (10 µM, n=8). The extract antispasmodic effect has been potentiated in the presence of glibenclamide, (* P<0.05, ** P<0.01, *** P<0.001).

Effect of L-NAME on extract antispasmodic activity

Figure 3 shows that incubation of ileum preparation (20 min) with L-NAME (100 μM, n=7), as a nitric oxide synthase inhibitor, did not reduce the TLE (0.125-2 mg/ml) spasmolytic effect on ileal contractions induced by KCl (60 mM).

Effect of naloxone on extract antispasmodic activity

incubation of ileum preparation (30 min) with naloxone (1 μM, n=8) as a non-selective opioid receptor antagonist did not alter the TLE (0.125-2 mg/ml) spasmolytic effect on KCl-induced ileal contraction, as shown in Figure 4. This antagonist however increased the extract spasmolytic effect at 0.5 mg/ml ($P<0.05$).

Extract antispasmodic activity on $CaCl_2$-induced contractions in ileum

Applying cumulative concentrations of $CaCl_2$ to the Ca^{2+}-free and high potassium (60 mM) Tyrode solution caused ileal contractions dose-dependently ($P<0.0001$, n=7). However, after tissue incubation with different concentrations of TLE (3 min, 0.25-2 mg/ml), the spasmogenic activity of $CaCl_2$ was attenuated significantly as is shown in figure 5. The spasmogenic effect of $CaCl_2$ at 2.7 mM was considered as 100% contraction. Statistical analysis indicates that the spasmolytic effect of the extract at 0.5 mg/ml. ($P<0.05$-$P<0.001$).

Effect of glibenclamide on the extract antispasmodic activity

incubation of ileum with glibenclamide (10 μM, 5 min) as an ATP-dependent potassium channel blocker not only did not reduce the TLE spasmolytic activity on CCh (10 μM)-induced ileal contraction but rather potentiated this effect (n=14, $P<0.05$-$P<0.001$) as is shown in figure 6.

Effect of tetraethylammonium on the extract antispasmodic activity

incubation of ileum with tetraethylammonium (1 mM, 5 min) as an calcium-operated potassium channel blocker not only did not reduce the TLE antispasmodic effect on carbachol-induced ileal contraction but rather increased this effect (n=9, $P<0.01$-$P<0.001$) as shown in figure 7.

DISCUSSION

In the present study it is shown that *Tecoma stans* (L.) Juss hydroalcoholic leaf extract (TLE) reduced the rat ileum contractions induced by KCl and CCh probably through voltage dependent calcium channels (VDCCs). The reduction in ileal contraction during these experiments was not because of tiredness of the ileum smooth muscle since recording contractions induced by KCl and CCh for 25 min did not show any reduction. On the other hand, the extract spasmolytic activity is occurred on the smooth muscle membrane because this effect was disappeared by washing the tissue and refreshing the organ bath solution and ileum responsive to spasmogens were returned to normal. If the TLE could induce its effect after entering in to the cells, by refreshing the bath solution this effect should not be removable.

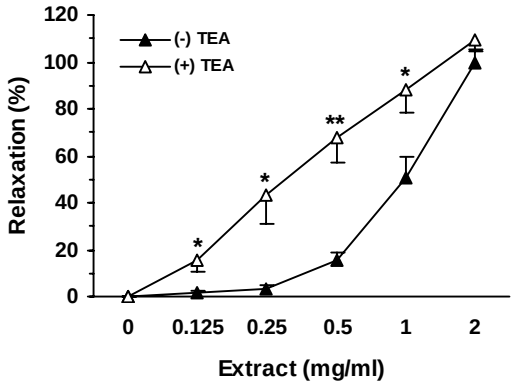

Figure 7. Spasmolytic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileal contractions induced by CCh (10 μM, n=14) before (-) and after (+) tissue incubation (5 min) with tetraethylammonium (TEA, 1 mM, n=9). The extract antispasmodic effect has been potentiated in the presence of TEA (* $P<0.01$, ** $P<0.001$).

Elevating intracellular calcium level is a key factor to control gastrointestinal smooth muscle tension (17) and high potassium concentration depolarized smooth muscle cells, activates voltage dependent calcium channels (18) which in turn increases $[Ca^{2+}]_i$ and causes contraction. The presence of L-type VDCCs has been reported in rat ileum (19). Carbachol (CCh), as a cholinergic agonist which is resistant to acetylcholinesterase (20), binds to M_2 and M_3 receptors and contracts ileum (21) by promoting calcium influx via receptor-operated calcium channels (22). In addition, CCh promotes inositol triphosphate synthesis (IP_3) via phospholipase C activation which in turn increases calcium release from intracellular calcium pools i.e. sarcoplasmic reticulum (23). These spasmogens (KCl as a non-receptor and CCh as a receptor operating spasmogen), in the same way elevates $[Ca^{2+}]_i$ and initiate contraction. It seems therefore that the TLE, somewhat, disturbs the calcium influx. This suggestion is supported by the extract antispasmodic effect on the $CaCl_2$-induced contractions in Ca^{2+}-free but high potassium Tyrode solution as presented in the result section.

In supporting this hypothesis, it has been suggested that those substances that inhibit KCl-induced contraction in smooth muscle, induce their effect by blocking VDCCs (24). It has been reported that, extracellular high K^+ concentration depolarized the cells (12) but a sufficient $[Ca^{2+}]_i$ is required to initiate contraction (22). Our results showed that the extract inhibited the $CaCl_2$-induced contractions therefore calcium channels possibly were involved. There is no possibility to assume that the extract has an anticholinergic activity because in this case the extract could only inhibit CCh-induced contractions but not the KCl-induced contractions. Since β-adrenoceptors activation relaxes ileum (25) the ineffectiveness of propranolol indicate that these receptors were not involved. Nitric oxide relaxes ileum via increasing cGMP synthesis (26) but the extract inhibitory effect was unaffected by L-NAME, as a nitric oxide synthase inhibitor, which indicates that at least, NO production was not involved in the extract activity. It is documented that opioid receptors activation relaxes ileum (27), however, the ineffectiveness of naloxone, as a non-selective opioid antagonist, indicated that these receptors were involved neither. This part of our results was consistent with the report that tecostanine of *Tecoma stans* has no interaction with opioid receptors (7). Another mechanism that could be involved was activation of potassium channels by the extract. Glibenclamide as an ATP-dependent K^+ channel blocker (28) and tetraethylammonium (TEA), as a calcium-operated K+ channel blocker (29, 30) were unable to reduce the extract spasmolytic effect. Therefore, these channels were not involved neither. Although, it has been reported that TEA is a non-selective K^+ blocker (31), this report seems would not alter the reputation. In our knowledge, the antispasmodic effect of *Tecoma stans* (L.) Juss on other smooth muscles has not been reported so far, therefore, comparison of our results with other investigations was impossible. The significant spasmolytic effect of *Tecoma stans* leaf extract and prescribing this plant as a traditional medicine in Latin America may suggest that other pharmacological studies of this plant might be valuable.

ACKNOWLEDGEMENT

The authors wish to thank the Ahwaz Jundishapur University of Medical Sciences for supporting this work financially and to thank Dr. Siahpoosh and Dr. Khaleghi for the plant identification.

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