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Effects of Kreysigine, an Alkaloid Isolated from *Colchicum decaisnea* on Ileum Smooth Muscle and Intestinal Motility of Rats

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Abstract: Effects of kreysigine isolated from *Colchicum decaisnea* Boiss on rat ileal smooth muscles and intestinal motility were studied. Kreysigine caused a significant dose dependent relaxant effect (EC_{50} 5.7×10^{-5} M) on rat ileal smooth muscles. The relaxant effects of the kreysigine were not affected by pretreatment with propranolol, but were significantly attenuated by methylene blue pretreatment. Pretreatment with theophylline significantly potentiated the inhibitory effect of kreysigine. These observations suggest that the relaxant effect of kreysigine on ileal smooth muscle could be mediated through the elevation of intracellular cyclic nucleotides and eventually decreasing cytosolic calcium concentration. Kreysigine also caused a significant decrease in propulsion of gastrointestinal motility. This study demonstrates that kreysigine may be a useful agent for the treatment of gastrointestinal disorders, such as diarrhea.

Key words: *Colchicum decaisnea*, kreysigine, ileum, intestinal motility, relaxation, rat

INTRODUCTION

Colchicum (Liliaceae) is a toxic plant to most animals and humans (Yamada *et al*., 1998; Brvar *et al*., 2004). It grows well in Jordan and in other Mediterranean countries (Zohary and Feinbran, 1966; Al-Mahmoud *et al*., 2006) that makes the plant relatively popular among Jordanian people. There are five *Colchicum* species in Jordan. These are *C. crocifolium* Boiss, *C. decaisnea* Boiss, *C. triphyllum* G. Kunze, *C. Steveni* Kunth and *C. ritchii* R. Br (Al Eisawi, 1982; Al-Mahmoud *et al*., 2006). Due to the toxicity of this plant, its use in folk medicine is restricted only to treat acute gout and familial Mediterranean fever. However, several compounds with a potential medicinal values can be isolated from this plant. Several alkaloids have been isolated from *Colchicum decaisnea* (Abu Zarga, 1995; Abu Zarga and Sabri, 1991). Kreysigine ($C_{22}H_{27}NO_5$) is one of them. Few pharmacological studies on such compounds have been reported (Abu Zarga *et al*., 1992). This study was undertaken to evaluate the effects of kreysigine on isolated ileal smooth muscles of the rat and on the intestinal motility.

MATERIALS AND METHODS

Animals: Albino rats of either sex weighting 200-230 g bred in the animal house unit in the Faculty of Art and Science at Hashemite University were used. All animals used in this study were cared for in accordance with Hashemite University animal care committee regulations.

Ileal preparation: Rats were lightly anaesthetized with ether and were sacrificed by a sharp blow to the head and the abdomen was opened. Segments of the ileum about 1-2 cm long were removed and dissected free of adhering mesentery. The lumen was flushed with Physiological Salt Solution (PSS). The tissue was mounted in 10 mL organ bath containing PSS at 37±1°C and aerated with air (95% O_2 and 5% CO_2). A tension of 1 g was applied. The responses were recorded isosmetrically on a minigraph (Lafayette instrument company) after a 60 min equilibrium period during which the PSS was changed every 15 min as a precaution against tissue metabolites (Abu Ghalyun *et al*., 1997). After equilibrium period, kreysigine, propranolol, methylene blue, theophylline, or 0.1% DMSO was added directly to the organ bath. The responses of the ileum to kreysigine were expressed as a percentage of the maximum relaxation to papaverine (10^{-3} M) which was added at the end of the experiment.

Small intestine transit: 0.5 mL of charcoal food (5 g of activated charcoal suspended in 50 mL PSS) was given to five groups of six animals each after 18 h fasting but with free access to water. In group 1 and 2 the charcoal food was administrated to animals intragastrically 60 min after

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the intraperitoneal injection of kreysigine. Group 3 was treated with 0.3 mg/rat of atropine sulphate instead of kreysigine. Controls (group 4) were not treated with the drug but with its solvent (0.1% DMSO) before receiving the charcoal food intragatically. Group 5 was treated with PSS.

The animals were killed after 60 min of charcoal administration and the small intestine, from the pylorus to the caecum, was rapidly removed and laid out on white filter paper for inspection and measurement of the distances traversed by the front of the charcoal food. This distance was calculated as a percentage of the whole intestine length.

Acute toxicity test: The LD_{50} of kreysigine was determined in mice using the method of Lorke (1983).

Solutions: Physiological salt solution was prepared daily and had the following composition (mM): 118.00 NaCl, 4.70 KCl, 25 $NaHCO_3$, 1.00 NaH_2PO_4 H_2O, 0.50 Na_2HPO_4, 11.10 glucose, 2.50 $MgCl_2$. $6H_2O$ and 2.50 $CaCl_2$. $2H_2O$. Kreysigine with purity of 90% (determined by thin layer chromatography (TLC) and NMR) was dissolved in DMSO (0.1%). The stock solution was kept refrigerated until shortly before use and dilutions were made with PSS and warmed to 37°C. Propranolol, methylene blue, theophylline and atropine sulphate were prepared by dissolving them in PSS.

Statistical analysis of the data: All values were expressed as the mean±SE and student's t-test was used to determine the significance of the different means. Values of $p<0.05$ were considered statistically significant. The effective concentration producing 50% of the maximum response (EC_{50}) was obtained by the best visual fit from the plot of the individual experiments.

RESULTS

Relaxant effect of kreysigine on isolated ileum: In concentrations ranging from (10^{-6}-10^{-3} M) kreysigine caused a concentration-dependent decrease in the amplitude of the contractions and relaxed the tone of the longitudinal segments of the ileum (Fig. 1). The EC_{50} of kreysigine for relaxation of ileal segments was 5.7×10^{-5} M. The relaxant effect of kreysigine was fully reversible in all cases, following washout of the preparations. The relaxation was not affected by propranolol (3×10^{-7} M) but attenuated significantly by methylene blue (10^{-3} M) (Fig. 1). Pretreatment with theophylline (10^{-5} M) significantly potentiated the inhibitory effect of kreysigine (Fig. 2). A solution of 0.1% DMSO showed no significant effect on rat ileal smooth muscles.

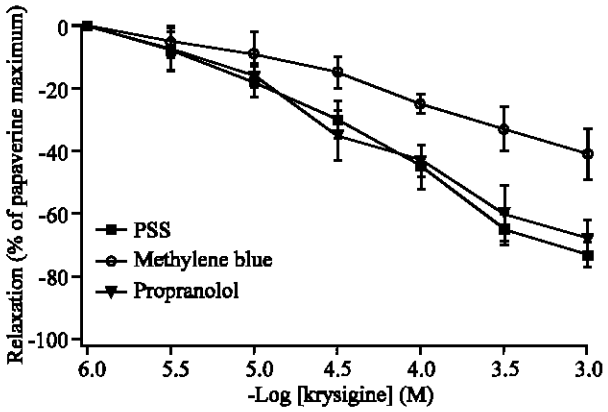

Fig. 1: Concentration-effect curve of kreysigine on rat isolated ileum in the presence of PSS, propranolol or methylene blue. Values are mean±SE of six experiments. ($p<0.05$) (Student's t-test)

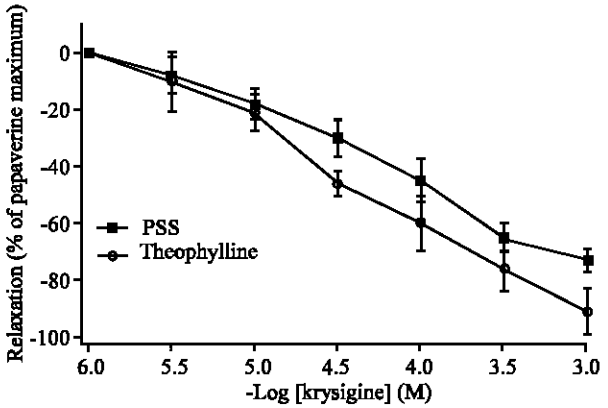

Fig. 2: Concentration-effect curve of kreysigine on rat isolated ileum in the presence of PSS or theophylline. Values are mean±SE of six experiments. ($p<0.05$) (Student's t-test)

Table 1: Effect of kreysigine on intestinal motility expressed as distance traveled by the charcoal food as % of the total intestinal length

Treatment	Dose	Movement of charcoal meal (%)	% of inhibition
Control (PSS)	2 mL kg^{-1}	67.22±6.27	-
Control (0.1% DMSO)	2 mL kg^{-1}	65.30±4.10	2.86
Atropine sulphate	0.3 mg/0.5 mL/rat	29.30±3.50*	56.40
Kreysigine	1 mg/0.5 mL/rat	53.60±5.30*	20.26
Kreysigine	3 mg/0.5 mL/rat	41.10±3.20*	38.85

Values are mean±SE of six experiments, * Significantly different from control: ($p<0.05$) (Student's t-test)

Kreysigine reduces small intestinal transit: Kreysigine significantly inhibited the propulsion of the charcoal meal through the small intestine with respect to the control group. The highest inhibition of intestinal transit was obtained with atropine sulphate. The results are shown in

Table 1. The preliminary acute toxicity test in mice established the LD_{50} to be 40 mg kg^{-1} body weight.

DISCUSSION

Relaxant effect of kreysigine on isolated ileum: Kreysigine has been found to cause a concentration dependent inhibition of the contraction of the rat isolated ileum. These observations are consistent with the reported effects of many other alkaloids, such as rutaecarpine thaliciminine, oblongine chloride and allocryptopine on ileal smooth muscles (Chiou *et al*., 1994; Abdalla *et al*., 1991, 1993; Abu-Ghalyun *et al*., 1997). The smooth muscles contractions are known to depend on the concentration of either extracelluar and/or intracellular Ca^{+2} (Jiang and Stephens, 1994; Ganitkevich *et al*., 2002; Matthew *et al*., 2004). Since kreysigine caused an inhibition of the smooth muscle contractions of the ileum, this implies that this alkaloid decreases the cytosolic calcium, either by inhibiting the Ca^{+2} influx or by inhibiting Ca^{+2} release from intracellular stores, or both.

Moreover, it is known that agents that elevate the level of cyclic nucleotides induce relaxation of smooth muscles (Karsten *et al*., 2003; Kang *et al*., 2002). Many alkaloids, such as berberine, allocryptopine and atherosperminine were found to elevate intracellular cyclic nucleotides through inhibition of phosphodiesterase (Kang *et al*., 2002; Abu Ghalyun *et al*., 1997; Lin *et al*., 1993), the enzyme controlling the cellular level of cAMP and cGMP. Since the relaxant effect of the alkaloid kreysigine resembles that of the above alkaloids, the inhibition of phosphodiesterase and the eventual increase of the cellular concentration of cyclic nucleotide offers a good basis for explanation of some observed effects of kreysigine on ileal smooth muscle.

The above suggestion of the mechanisms of action of kreysigine was investigated by studying its effect in the presence of methylene blue, an inhibitor of the activation of soluble guanylate cyclase, preventing the rise in cGMP (Hyman *et al*., 1991) and theophylline, an inhibitor of phosphodiesterase, increasing the level of cyclic nucleotides (Ruiz *et al*., 1988). Methylene blue decreased the maximum inhibitory effect of kreysigine on the ileal preparations, supporting the conclusion that this alkaloid may act by elevating the intracellular level of cGMP.

The results obtained from experiments with theophylline also support the conclusion that this alkaloid acts by inhibiting phosphodiesterase enzyme and elevating cellular cyclic nucleotides, which causes relaxation through many proposed mechanisms. These mechanisms includes 1) reducing Ca^{+2} influx by activating K^{+} channels, which Induces hyperpolarization and decreasing Ca^{+2} influx through L-type Ca^{+2} channels; 2) modulation of the sensitivity of contractile apparatus to existing concentrations of Ca^{+2} (Savineau and Marthan, 1997); 3) inhibition of the binding of Ca^{+2} to the contractile proteins; 4) inhibition of phosphoinisitol hydrolysis, since inositol 1,4,5 triphosphate, an intermediate product of receptor-mediated phosphatidylinositol, 4, 5 biphosphate hydrolysis, has been shown to release Ca^{+2} from intracellular stores in smooth muscle cell.

Propranolol did not significantly affect the relaxant effect of kreysigine on ileal smooth muscles. These data suggest that this alkaloid did not act through activation of adrenergic receptors and indicate that kreysigine shows specific sites of action (Wanajo *et al*., 2004; Yamanishi *et al*., 2003).

Kreysigine reduces small intestinal transit: Kreysigine was found to inhibit the transient time of the small intestine contents. This inhibitory effect of kreysigine was more pronounced at high concentration (3 mg/0.5 mL/rat) than at low concentration (1 mg/0.5 mL/rat). These observations are consistent with the reported effects of many other alkaloids, such as morphine, atropine, anisodine, hyoscine butylbromide and scopolamine on intestinal motility (Jiao *et al*., 2002; Pan and Han, 2004). Since the intestinal motility can be inhibited by many regulatory mechanisms such as hormones, the enteric nervous system and autonomic nervous system (sympathetic) (Jiao *et al*., 2002; Smout; 2004; Kiely *et al*., 2005; Hansen, 2003a, b) kreysigine may decrease the small intestine transient by these mechanisms. Further studies are required to ascertain the precise mechanisms of kreysigine on intestinal motility. Whatever the mechanism of action of kreysigine, our study suggests that kreysigine could be a useful agent for ileal motor dysfunction, such as diarrhea.

In conclusion, kreysigine has been shown to exert relaxant effects *in vitro* on the isolated ileal smooth muscles and reduced the intestinal transient *in vivo*.

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