

Effect of toxin- γ from *Tityus serrulatus* scorpion venom on gastric emptying in rats

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Abstract

The effect of toxin- γ from *Tityus serrulatus* scorpion venom on the gastric emptying of liquids was studied in 176 young adult male Wistar rats (2-3 months of age) divided into subgroups of 8 animals each. Toxin- γ was injected *iv* at doses of 25, 37.5, 50 or 100 $\mu\text{g}/\text{kg}$ and the effect on gastric emptying was assessed 30 min and 8 h later. A time-course study was also performed by injecting 50 μg of toxin- γ/kg and measuring the effect on gastric emptying at times 0.25, 0.5, 1, 2, 4, 8, 24 and 48 h post-venom. Each envenomed animal was paired with its saline control and all received a saline test meal solution containing phenol red (60 $\mu\text{g}/\text{ml}$) as a marker. Ten minutes after administering the test meal by gavage the animals were sacrificed and gastric retention was determined by measuring the residual marker concentration of the test meal. A significant delay in gastric emptying, at 30 min and 8 h post-venom, was observed only after 50 and 100 μg of toxin- γ/kg compared to control values. The responses to these two doses were significantly different after 8 h post-venom. Toxin- γ (50 $\mu\text{g}/\text{kg}$) significantly delayed the gastric emptying of liquids at all times studied, with a peak response at 4 h after toxin administration compared to control values. These results indicate that the *iv* injection of toxin- γ may induce a rapid, intense and sustained inhibition of gastric emptying 0.25 to 48 h after envenomation.

Key words

- Gastric emptying
- *Tityus serrulatus* scorpion venom
- Toxin- γ

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Presented at the 12th World
Congress on Animal, Plant and
Microbial Toxins, Cuernavaca,
Mexico, September 21-26, 1997.
Research supported by FAEP-FCM-
UNICAMP (No. 569-96). Publication
supported by FAPESP.

Received May 18, 1998
Accepted January 15, 1999

Envenomation by scorpions is a serious health problem in Brazil, especially in the south-eastern region, where *Tityus serrulatus* has been considered to be the most dangerous species, responsible for most of the accidents (1,2). Among the clinical features observed in severely envenomed patients, mainly children, gastrointestinal symptoms such as vomiting, excessive salivation and

abdominal pain have been frequently reported (1,2). Based on these and other clinical observations, several studies have been carried out to improve our understanding of the effects of scorpion venom on the gastrointestinal system.

Experimental studies have shown that the injection of whole venom and purified toxins from the venom of *Tityus serrulatus* and

other scorpions can cause profuse salivation (3), increased gastric (4,5) and pancreatic (6) secretion, acute gastric mucosal (7) and pancreatic (1,6) injuries, as well as disorders of intestinal motility (1). Most of these effects have been related to the acute autonomic disturbances triggered by the venom, which can provoke both the activation and delayed inactivation of neuronal sodium channels, neuronal cell depolarization and the release of acetylcholine and catecholamines (1).

Since the contractile state of gastric smooth muscle cells depends on a balance between excitatory (cholinergic) and inhibitory (adrenergic, dopaminergic, non-adrenergic and non-cholinergic vagal) chemical messages (8,9), the present study was designed to investigate the effect of the major toxic protein purified from *T. serrulatus* scorpion venom, toxin- γ (10), on gastric emptying in rats given a physiological saline test meal.

The study was conducted on 176 young adult male Wistar rats (2-3 months of age), weighing 216-326 g, divided into subgroups of eight animals each. The animals were acclimatized to the laboratory for at least seven days before the experiment. During that time they received standard rat chow (Labina, Purina) and water *ad libitum*. Before the test day, the rats were transferred to individual cages and subjected to a 20-h fasting period during which only water was available.

Prior to measuring gastric retention, the animals received an *iv* injection (via a tail vein) of either 0.9% saline (control animals) or toxin- γ dissolved in 0.9% saline, at doses of 25, 37.5, 50 or 100 $\mu\text{g}/\text{kg}$ at respective concentrations of 12.5, 18.75, 25 and 50 $\mu\text{g}/\text{ml}$, and the effect on gastric emptying was assessed 30 min and 8 h later. A time-course study was performed by injecting 50 μg of toxin- γ/kg and measuring the effect on gastric emptying at times 0.25, 0.5, 1, 2, 4, 8, 24 and 48 h post-venom. Samples of toxin- γ

were provided by C. Chavéz-Olórtegui (FUNED, Belo Horizonte, MG, Brazil).

Each envenomed animal was paired with its saline control and all received a saline test meal solution by orogastric infusion in a volume of 2 ml/100 g animal weight containing phenol red (60 $\mu\text{g}/\text{ml}$) as a marker. Saline solution has been considered a suitable test meal to evaluate gastric emptying without intestinal interference since there is no activation of intestinal receptors, in contrast to that obtained with nutritive test meals (8). The techniques used for orogastric intubation and for obtaining the residual contents were essentially those described by Belangero and Collares (11) and Bucarechi and Collares (12). Ten minutes after receiving the test meal by gavage the animals were sacrificed and gastric retention was determined by measuring the residual marker concentration in the test meal.

The significance of the differences in gastric retention between subgroups was evaluated using nonparametric statistical methods. The Mann-Whitney U-test was performed when $k = 2$ ($P < 0.05$). When $k > 2$, the Kruskal-Wallis test ($P < 0.1$) and Mann-Whitney U-test corrected for multiple comparisons ($P < 0.01$) were also used. The results are shown as box and whisker plots in the figures. In each plot, the upper and lower short horizontal lines indicate the maximum and minimum gastric retention values observed, respectively. The median and first and third quartiles of the gastric retention values for each group are reported as the intermediate, lower and upper horizontal lines which are used to construct each rectangle, respectively.

A significant delay in gastric emptying was observed only after 50 and 100 μg of toxin- γ/kg . In contrast, the responses to these two doses were significantly different after 8 h post-venom (Figure 1A and B).

Toxin- γ (50 $\mu\text{g}/\text{kg}$) induced a rapid, intense and sustained increase in gastric retention of the saline test meal, since significant

differences between the envenomed groups and their respective controls were observed as early as after 15 min and up to 48 h, with a peak response at 4 h post-venom injection (Figure 2).

Although we did not study them here, we may assume that several mechanisms are involved in the increase in gastric retention observed in the envenomed animals.

As is the case for scorpion venoms, *Phonetreria nigriventer* spider venom, which also acts on neuronal sodium channels, may provoke the release of catecholamines and delay gastric emptying in rats (12). According to Burks (13), adrenergic receptors in gastric smooth muscle cells are essentially beta-adrenergic, and agonists for these receptors have been shown to inhibit gastric emptying in man (14).

Sofer et al. (15) reported that *Leiurus quinquestriatus* scorpion venom provoked gastrointestinal ischemia in pigs whereas Gwee et al. (16) and Teixeira et al. (17), respectively, demonstrated that *L. quinquestriatus* and *T. serrulatus* scorpion venoms were capable of relaxing rat isolated anococcygeus muscle and rabbit isolated corpus cavernosum via the local release of nitric oxide. These observations are important since a decrease in gastrointestinal blood flow (18) and the local release of nitric oxide (19) may inhibit gastric smooth muscle cell activity. Additional studies are required to evaluate these possibilities.

The increase of gastric acid secretion may also alter gastric emptying (8). Troncon et al. (20) reported an increase in gastric secretion and a significant delay in the gastric emptying of a saline test meal in rats treated with tityustoxin from *T. serrulatus* scorpion venom. These results indicate that the delay in gastric emptying was not influenced by an increase in gastric acid secretion since a similar response was also observed in envenomed rats pretreated with ranitidine (20).

The sustained increase in gastric reten-

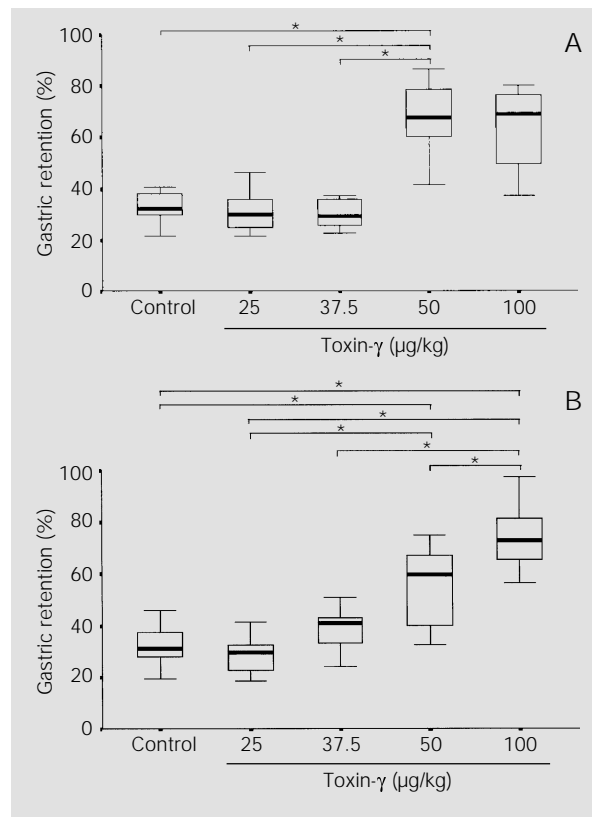


Figure 1 - Effects of dose of toxin- γ from *Tityus serrulatus* scorpion venom on the gastric retention of a saline test meal in rats 30 min (A) and 8 h (B) after iv administration of the toxin. The data are shown as box and whisker plots for 8 rats/subgroup. *P<0.01 for the comparisons indicated at the top of each panel (Kruskal-Wallis test and Mann-Whitney U-test corrected for multiple comparisons).

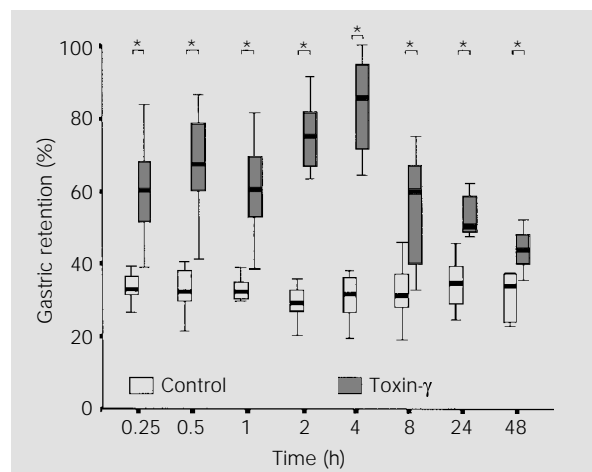


Figure 2 - Duration of the effect of toxin- γ from *Tityus serrulatus* scorpion venom on the gastric retention of a saline test meal from 0.25 to 48 h after iv injection of 50 μg of toxin/kg. The data are shown as box and whisker plots for 8 rats/subgroup. *P<0.05 for the comparisons indicated at the top of the figure (Mann-Whitney U-test).

tion of the saline test meal induced by toxin- γ was another interesting observation (Figure 2). This effect may be partially related to the very high affinity (dissociation constant of 1×10^{-11} in frog muscle) of this toxin for site IV of the sodium channels (21).

In conclusion, the present data indicate that the *iv* injection of toxin- γ induces a rapid, intense and sustained inhibition of gastric emptying. Since *T. serrulatus* scorpion venom may increase gastric secretion (4,5) and cause acute gastric lesions (7) as well as gastric emptying disturbances in ex-

perimental animals, a higher risk of acute gastric mucosal injuries should be considered in cases of severe human envenomation by this species.

Acknowledgments

We thank Prof. Dr. Eliana M. Barison (Departamento de Pediatria, FCM, UNICAMP) for technical assistance and Prof. Dr. Stephen Hyslop (Departamento de Farmacologia, FCM, UNICAMP) for reviewing the English text.

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