Effects of gonyleptidine on the orb-web spider *Araneus lathyrinus* (Holmberg, 1875)

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Summary

Harvestmen possess several chemical substances as a defence against predators. Among these, gonyleptidine has been one of the most studied and best characterized. This substance, produced by the harvestman *Acanthopachylus aculeatus*, is composed mainly of noxious substances such as benzoquinone. However, little is known about the secondary effects caused by the ingestion of this substance. Web-building spiders are an appropriate group to test the effects of several neurotoxic compounds, since the effects of these substances are directly reflected in their behaviour, notably in web construction. The aim of this study was to analyse the influence of the ingestion of gonyleptidine on spider web building, using as a model the orb weaver *Araneus lathyrinus*. We designed two experimental groups composed of subadult specimens. In one we offered larvae of *Tenebrio molitor* as prey for the spider; each mealworm was contaminated with 2.0 µl of gonyleptidine. In the control group the spiders were fed with uncontaminated *T. molitor* larvae. We found adverse effects on the group of spiders contaminated with the gonyleptidine, such as the construction of irregular webs, and loss of predatory capability. These results suggest that, when ingested, the gonyleptidine deteriorates the spider's coordination, similar to the effect of neurotoxic substances, like some pesticides. Our findings indicate that gonyleptidine has not only a repellent–defensive function but also a toxic effect on predators.

Introduction

During the last few years, there has been an increasing interest in evaluating the effect of chemical substances on the nervous systems of animals and humans (O'Brien, Kaneene & Poppenga 1993). Traditionally, most studies have been biased towards mammals, due to their greater resemblance to humans (Ballatori & Villalobos 2002). Nevertheless, there has been an increase in the use of non-mammalian animals, particularly invertebrates, in neurotoxicological research (Peterson *et al*. 2008). These organisms present some traits, such as a simple nervous system, well known metabolic and physiological pathways, and lack of restrictions related to manipulation and experimentation, which make them suitable models for toxicological investigation (Salanki 2000).

Among invertebrates, the spiders have proved to be an important group to test the effects of several toxicological compounds (Haynes 1988) since the effect of these substances is reflected directly on spider behaviour (Benamú *et al*. 2013; Pekár 2013). Orb-weaving spiders, for example, suffer important alterations in their web-building behaviour when they are exposed to the effect of different toxins; these shifts include weaving irregularly shaped webs and abnormal radii and spirals (Witt 1971). For example, Witt & Reed (1965) studied spider sensitivity to the intake of several substances and their specific effect on web-building behaviour, concluding that treated spiders built fragmentary webs due to the influence of drugs on spider physiology. Hesselberg & Vollrath (2004) found a similar pattern in *Araneus diadematus*, where the spiders which had consumed substances such as caffeine and amphetamine modified the web geometry and building frequency. Similarly, Benamú *et al*. (2010) showed that spiders treated with glyphosate showed a significant decrease on the number of radii on the web.

Harvestmen are known for producing volatile secretions commonly used for communication, including alarm and intraspecific recognition; nevertheless, they act mainly as defence mechanisms. The secretion produced by harvestmen often include a cocktail of repellent substances which contain noxious compounds as benzoquinones, ketones, and phenols (Eisner *et al*. 1977; Rocha *et al*. 2013), creating a chemical shield which deters several potential predators such as some spiders, ants, and some vertebrates (Machado *et al*. 2005). The chemical properties of these secretions have been extensively studied in several species and gonyleptidine is one of the best known (Rocha *et al*. 2013). This substance, present in the harvestman *Acanthopachylus aculeatus* (Kirby, 1819), is comprised mainly of 1-4 benzoquinone. It plays an important role in the defence of *A. aculeatus*, acting as a deterrent for several predators, such as ants; nevertheless, it has proved to be poorly effective against some other predators, such as wolf spiders (Eisner, Rossini & González 2004).

In spite of the great number of studies focused on the repellent and chemical properties of defensive secretion in harvestmen, little is known about the secondary effects caused by the ingestion of this substance. The aim of this paper is to describe the effects after ingestion of gonyleptidine, using as model the spider *Araneus lathyrinus* (Holmberg, 1875), an orb-weaving spider commonly found in Uruguayan native forests. Although *A. aculeatus* harvestmen are not natural prey of these spiders, we considered them a suitable model to test the effects of gonyleptidine after ingestion because these would be easily reflected by the spider's behaviour, particularly web building. If the spider's web shows alterations after the ingestion of gonyleptidine, then it would show that this substance could have not only irritant but also noxious properties for animals which feed on this harvestman species.

Methods

Specimen sampling

We collected subadult females of *Araneus lathyrinus* and adult females of the harvestman *Acanthopachylus aculeatus* in Montevideo, Uruguay (34°50′16.69″S 56°25′07.97″W). After collection, each spider was kept isolated in a glass container of 13 cm height and 9 cm in diameter, with water and sticks to support the web, and covered with a mesh, in laboratory conditions. Spiders were fed *ad libitum* with

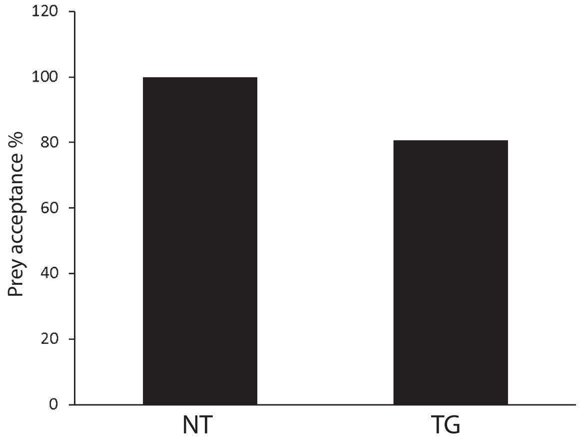

Fig. 1: Prey acceptance of the spider *Araneus lathyrinus* on *Tenebrio molitor* non-treated (NT) and treated (TG) with gonyleptidine.

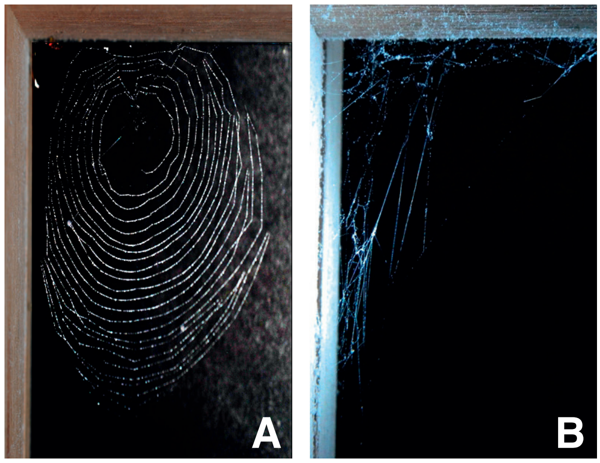

Fig. 2: Webs rebuilt by the spider *Araneus lathyrinus* after feeding on *Tenebrio molitor*. **A** non-treated; **B** treated with gonyleptidine.

pieces of *Tenebrio molitor* Linnaeus, 1758 larvae and kept with a photoperiod of 12 hours L : D at a temperature of 25°C and 70% humidity. Five days before the trials, each spider was placed in a wooden box of 20 cm × 15 cm × 10 cm with two walls of sliding glass, to facilitate the web observation and photographic record.

Experimental design

In order to test the effect of the gonyleptidine on the web construction of *A. lathyrinus*, we created two experimental groups. In the first group, 18 subadult females were fed once with larvae of *Tenebrio molitor* previously contaminated with gonyleptidine. The substance was extracted directly from 10 adult *Acanthopachylus aculeatus* (0.91 ± 0.31 cm in body length) using a micropipette (2–20 µl), the extractions were mixed and then a volume of 2.0 µl was applied directly to the body of *T. molitor*. The extraction was made immediately before the experiment to assure the use of fresh substance. In the second group, we fed another 20 spiders once with non-contaminated *T. molitor* larvae. Since gonyleptidine is considered a repellent substance, in both groups we recorded the acceptance percent of contaminated and non-contaminated larvae. We destroyed the web after the consumption of the prey (only of those individuals which accepted the larvae); this procedure was applied to both groups. Based on previous observations, we left a period of 48 hours so the spider could reconstruct the web.

Afterwards, we counted the number of spiders which had rebuilt normal webs, including the elements necessary for prey capture, such as the radii and hub, and compared them with the spiders which had built webs without these elements. One *Drosophila* sp. fly was added to each spider which rebuilt the web in order to evaluate if treated and non-treated spiders were able to capture prey. Since our data corresponded mainly to counts, comparisons between the prey acceptances in both groups were made using a χ-square test. Comparison between the number of contaminated and non-contaminated spiders which rebuilt the web (normal and irregular webs) and the fly capture between both groups were made using the Fisher's exact test. All the statistical analyses were performed using the software R version 3.0.1 (R Project 2012).

Results

Spiders more frequently accepted the *Tenebrio molitor* without gonyleptidine than the *T. molitor* with gonyleptidine (Fig. 1); nevertheless, the groups were not significantly different ($\chi^2 = 2.346$, $P = 0.126$). All orb webs of *Araneus lathyrinus* fed with untreated *T. molitor* larvae showed the typical three-dimensional orb web, with signal and barrier threads (Fig. 2). The spiders which fed on contaminated *T. molitor* built irregular webs in all cases, with some of the threads lacking adhesive elements (Fig. 2), except one spider which rebuilt a full irregular web including spirals, hub, and radii. The number of rebuilt normal webs was more frequent (Fisher's exact test: $P < 0.01$) in non-contaminated spiders (Fig. 3). All of the spiders which fed on the larvae without gonyleptidine accepted the fly offered after rebuilding the web. All webs which missed the basic elements were unable to retain the offered *Drosophila* sp., so the fly consumption in the non-treated group was higher than the treated one (Fisher's exact test: $P < 0.01$).

Discussion

In the two experimental groups we obtained similar acceptance frequencies of *T. molitor* larvae, suggesting that our spiders were unable to recognize contaminated prey and were not inhibited to attack in spite of the repellent substance. These results were similar to those found in wolf spiders (Eisner *et al*. 2004), which accepted cockroaches contaminated with gonyleptidine.

Nevertheless, the ingestion of gonyleptidine caused negative effects on the spiders. Although all the spiders rebuilt their webs after the ingestion of gonyleptidine, they lacked some fundamental structures for prey retention (spirals, hub, and radii), preventing the spiders to capture and eat the offered prey. We could not assign any web structure to the long threads which lacked viscous elements rebuilt after the intoxication, since these threads did not show a defined

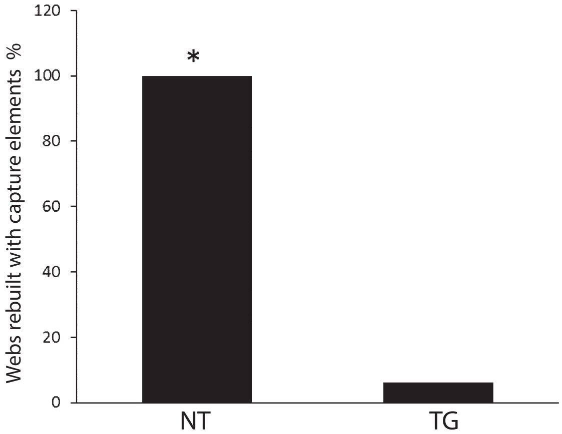

Fig. 3: Frequency of web reconstruction by the spider *Araneus lathyrinus* after feeding on *Tenebrio molitor* non-treated (NT) and treated (TG) with gonyleptidine.

position or shape by which we could determine whether they were marks or radii.

The specific action of the gonyleptidine upon the orb web of *A. lathyrinus* were similar to some results obtained in other researches where spiders were exposed to different toxins. Benamú *et al*. (2007) showed that two neurotoxical insecticides, endosulfan and spinosad, had negative effects on web rebuilding in *Araneus pratensis*, whose webs were irregular with alterations of the capture elements. Other substances can produce similar alterations to orb web constructions, like glyphosate (Benamú *et al*. 2010) and synthetic drugs (Witt & Reed 1965; Hesselberg & Vollrath 2004).

Consequently, we assume that, although gonyleptidine can be ingested by the spider, it affected the motor system of *A. lathyrinus* and, furthermore, we suggest that similar effects could be caused on other spiders which feed on harvestmen.

These results suggest that gonyleptidine could have a toxic and repellent effect on predators that could be caused by benzoquinones which can produce injuries to the nervous system (Chambers & Rowan 1982). This evidence suggests that opilionid secretions could be similar to those produced by some millipedes which also possess deterrent and noxious properties (Pasteels, Grégoire & Rowell-Rahier 1983; Eisner, Rossini & González 1998; Deml & Huth 2000). Although we did not test the mortality or other long-term effects caused by gonyleptidine on treated spiders, we suggest it can produce at least short-term effects on the motor system after ingestion, or other sublethal effects. Further studies should evaluate if these effects are also produced by the secretions of other harvestmen species, and the long term effects of these substances on natural enemies.

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