

CENTRAL AND PERIPHERAL NEUROTOXICITY INDUCED BY EXTRACTS AND FRACTIONS OF MANILKARA RUFULA IN COCKROACHES

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Resumo:

Plant secondary metabolites are applied in different areas, from the pharmaceutical, with the production of medicines, to agroindustrial, with their use as bioinsecticides. Vegetal species adapted to extreme environmental conditions, (low humidity and high temperatures), present a large diversity of secondary metabolites, being the Caatinga biome an example of extremist climates. The aim of this work was to evaluate the effect of crude extracts and enriched fractions of *Manilkara rufula*, a plant from the Caatinga, using the nervous system of cockroaches as a biological model. For this, adult *Nauphoeta cinerea* cockroaches of both sex, were used. The crude extract of *M. rufula* (CEMR) was administered at 25, 50 and 100 µg/g of animal and the methanolic (MFMR) and aqueous (AFMR) fractions were administrated at 10, 20 and 40 µg/g of animal. Also tested a octopamine neuromodulator (15 µg/g of animal) and phentolamine (0,01 µg/g of animal), an octopaminergic receptor antagonist. The neurolocomotor activity of the insects were tested, where the animals were monitored for 10 minutes with a webcam, and the records were analyzed by the software IDtracker® and Matlab ®. Behavioral grooming tests were performed, according to Sturmer et al. (2014). The neuromuscular activity was evaluated using the in vivo cockroach neuromuscular preparation (CNP), as described elsewhere (Martinelli et al. 2014). In neurolocomotion preparations, the CEMR (100 g/g of animal) led to a considerable decrease of the traveled route by the animals, consequently increasing, the number of episodes in which the animals remained immobile. Our data suggest that the extracts of *M. rufula* may be acting on octopamine, associated with rhythmic behaviors such as locomotion and cleaning. In behavioral tests of grooming the CEMR (50 g/g of animal) decreasing the leg cleaning behavior (controlled by the octopaminergic pathway) from 59±8 s/min (control) to 5±4 s/min (n=30). When octopamine was tested, the leg cleaning behavior was increased by 158±19 s/30 min. In contrast, phentolamine caused a decrease in leg grooming around 17±3 s/min. When CEMR (50 g/g of animal) was administered minutes after administration of octopamine, there was a decrease in leg grooming around 62±10 s/30 min. In CNP assays, all CEMR concentrations decreased contraction force of insects. The CEMR (50 g/g of animal) decreased contraction to 68±4% compared to control (101±1%; p 0,05). The neuromuscular junction is modulated mainly by Gamma-aminobutyric acid (GABA) and glutamate (GLU). The data suggest that extracts and fractions of *M. rufula* modulate the octopaminergic pathway, which in turn controls the release of GABA and GLU as an association neurotransmitter. The data obtained in this work demonstrate that the extracts of *M. rufula* have effect on the octopaminergic pathway of cockroaches, being the AFMR the main responsible for the observed effects, demonstrating the biotechnological potential of this species.

Palavras-chave: entomotoxic; nauphoeta cinerea; neurotoxicity; natural insecticides

Modalidade de Participação: Pesquisador

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CENTRAL AND PERIPHERAL NEUROTOXICITY INDUCED BY EXTRACTS AND FRACTIONS OF *Manilkara rufula* IN *Nauphoeta cinerea* COCKROACHES

1. INTRODUCTION

The synthesis of a secondary metabolite by a group of plants is related to adaptive features acquired during evolution (PICHESKY & GANG, 2000). Since the discovery of distinct groups and biological functions, the secondary compounds of plants are applied in several areas, from pharmaceuticals, to medicine production to agricultural and for development of novel insecticides (MEDEIROS, 2010).

Vegetal species adapted to extreme environmental conditions, (as low humidity and elevated temperatures) such as the Caatinga biome have a wide variety of secondary metabolites (GOMES et al. 2008). A plant found in this region is *Manilkara rufula*, belonging to the family Sapotaceae (PENNINGTON, 1990). Numerous studies are found in the genre *Manilkara*, but about the species *M. rufula* few results on biological action and phytochemical characterization are found. Stands out the antiparasitic activity presented by *M. rufula* against the parasite *Trichomonas vaginalis* (DE BRUM VIEIRA et al. 2016), demonstrating the potential of this plant.

There is a growing need for the use of insecticides of botanical origin, which are less toxic than conventional insecticides with no residual effects on the environment. In this sense, cockroaches are a viable model for studies of entomotoxicity, and the species *Nauphoeta cinerea* has been used successfully by our group, mainly due to its simplified nervous system (Sturmer et al., 2014). Considering the above, the objective of this study was to evaluate the activity of crude extract and enriched fractions of *Manilkara rufula* regarding the entomotoxic potential in cockroaches of the species *Nauphoeta cinerea*.

2. METHODOLOGY

Adult *Nauphoeta cinerea* (Olivier) cockroaches were used as experimental model. The crude extract of *M. rufula* (CEMR) and the metanolic (MFMR) and aqueous (AFMR) fraction were prepared at the following concentrations: 25, 50 e 100 e 10, 20 e 40 µg/g of animal. The compounds were previously dissolved in 0,5% of DMSO in water (Milli-Q). As biological activity assays exploratory behavior, tests were carried out, in the open field, as described by Adedara et al.(2015). In these trials it was evaluated *in vivo* locomotor activity of insects where they were monitored by a webcam (Philips, Brazil), for 10 minutes and the records later analyzed by software IDtracker® and Matlab®. Next, animals were subjected to *grooming* behavior tests, according to Sturmer et al. (2014). Electromyographic tests were also performed, which were mounted with anesthetized cockroaches by cooling, according to Martinelli et al. (2014). In these protocols were tested, in addition to compounds from *M. rufula*, pharmacological instruments such as octopamine neuromodulator (15 µg/g of animal) and phentolamine (0,01 µg/g of animal), an octopaminergic receptor antagonist.

All data were expressed as mean $\pm$ standard error (S.E.M), being they confronted by Student's "t" test or ANOVA, followed by the Tukey or Dunnett tests as *post hoc*.

3. RESULTS AND DISCUSSION

In general, the *M. rufula* extract and its subfractions induced significant behavioral changes in insects. The locomotor activity was significantly altered by the injection of CEMR, demonstrating a dose-dependent effect (Fig. 1). In these tests, doses of 50 and 100 $\mu\text{g/g}$ of animal were more effective in reducing locomotor activity (Fig. 1A). It was also observed an increase in the number of stops (n) events during the route of the cockroaches, corroborating with the reduction of the route carried out by the same. In the evaluation of these events, the control group was ($545 \pm 4 \text{ n/min}$) while administration of CEMR (100 $\mu\text{g/g}$ of animal) induced an increase in the immobility of the insects of $903 \pm 8 \text{ n/min}$ (Fig. 1B). The data obtained showed that CEMR (100 $\mu\text{g/g}$ of animal), induced lethargy in animals characterized by a stopping time of 83% of control ($n=30$, respectively) (Fig. 1C).

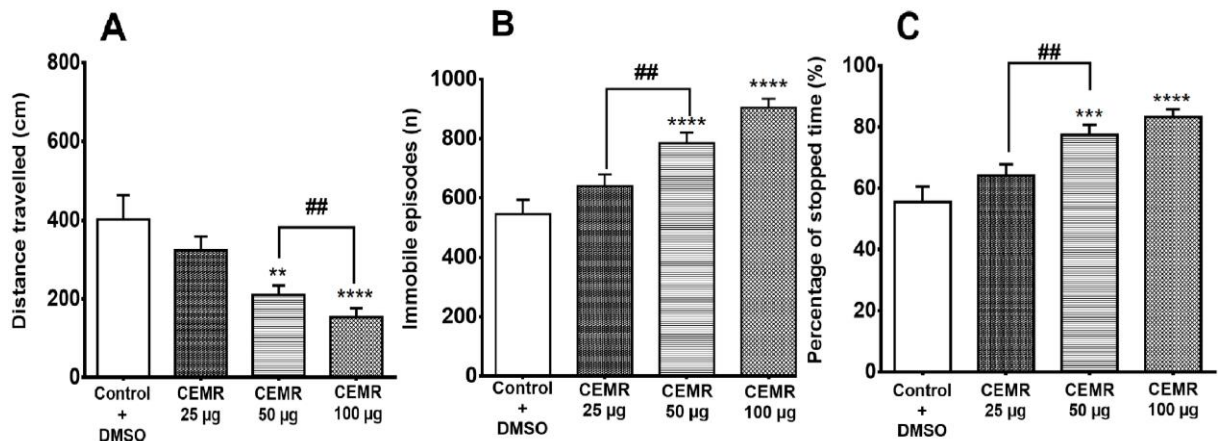


Figure 1: Decreased effect of CEMR on neurolocomotor activity in cockroaches of the species *Nauphoeta cinerea*. The author (2017).

In insects, rhythmic behaviors, such as walking and flying, are controlled by rhythmic central initiators, which start from the thoracic ganglia, which can be modulated by the neurotransmitter octopamine (MARDER, 2001). Thus, our data demonstrate that CEMR affects the locomotor activity of insects probably by the modulation of the monoaminergic insect system.

In addition, octopamine is a biogenic monoamine involved in several cell signaling in insects (ROEDER, 1999). For example, this monoamine is involved in the *grooming* activity, a common behavioral process in insects, related to hygiene behavior, mating and social interaction (WEISEL-EICHLER, 1999).

The administration of CEMR (25, 50 e 100 $\mu\text{g/g}$ of animal) induced a dose-dependent alteration in the grooming activity of the animals (Fig. 2). In these trials, changes were observed mainly in leg *grooming*, which is modulated primarily by the octopaminergic pathway. Thus, the analysis of the control records, with DMSO 0.5% was of ($59 \pm 8 \text{ s/min}$). When CEMR (50 $\mu\text{g/g}$ of animal) was tested the grooming time was ($5 \pm 4 \text{ s/min}$), ($n=30$, respectively for the control and treated) (Fig. 2A).

They also evaluated the effects of octopamine (15 $\mu\text{g/g}$ of animal) and phentolamine (0,01 $\mu\text{g/g}$ of animal), about *grooming*. When octopamine was assayed

there was an increase in leg *grooming* (158 ± 19 s/30 min), while phentolamine induced a decrease of leg grooming (17 ± 3 s/min). In trials where octopamine was administered prior to administration of CEMR (50 $\mu\text{g/g}$ of animal), there was a significant decrease in leg grooming behavior for 62 ± 10 s/30 min, compared to octopamine control (Fig. 2B). This latter data indicate that the octopaminergic route is involved in the reduction of grooming activity, induced by CEMR.

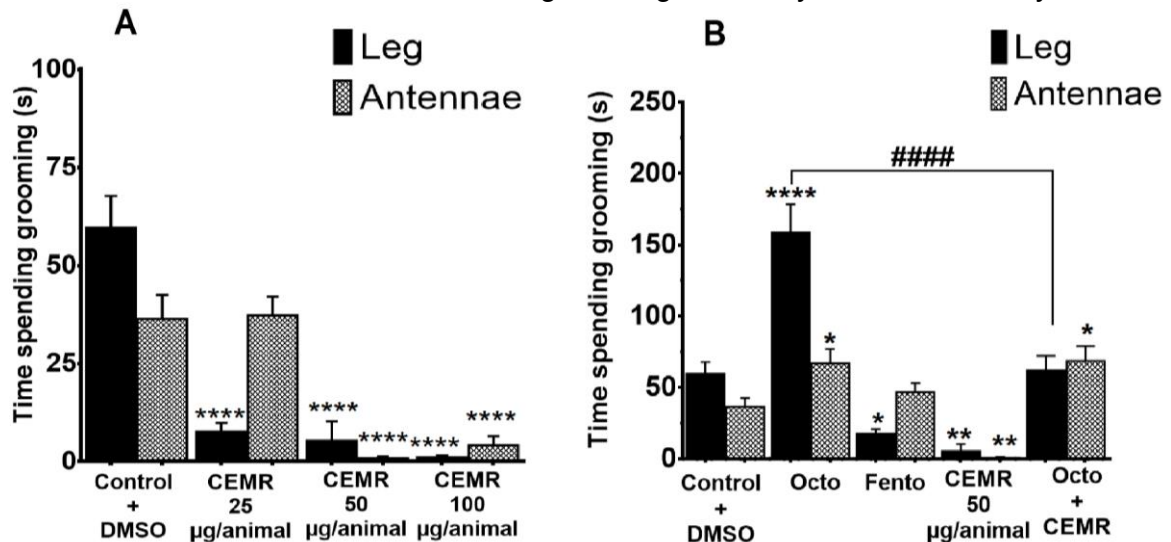


Figure 2: Effect of CEMR and pharmacological instruments on *grooming* behavior in cockroaches of the species *Nauphoeta cinerea*. The author (2017).

To prove a direct activity of CEMR on the neuromuscular junction of insects, the same was added to the *in vivo* neuromuscular preparation of cockroach *Nauphoeta cinerea* (CNP). Muscle contraction in insects is modulated by the release of glutamate (GLU - excitatory neurotransmitter) and gamma-aminobutyric acid (GABA - inhibitory neurotransmitter). Data indicate that octopamine may act as an association neurotransmitter, thereby controlling the release of GLU and GABA in the synaptic cleft (FAROOQUI, 2012).

In these assays, all concentrations of CEMR were effective in decreasing the amplitude of the muscular contraction force of the insects. The concentration of 50 $\mu\text{g/g}$ of animal of CEMR had a more prominent effect on the contraction amplitude, with $68 \pm 4\%$ decrease compared to control ($101 \pm 1\%$, $p < 0.05$) (Fig. 3A). This modulation may be related to an increase in GABA levels or a decrease in GLU levels. Considering the hypothesis that octopamine is the main route of action of CEMR, it is suggested that octopamine is acting as an association neurotransmitter during the neuromuscular contraction process, interfering with the excessive release of GABA or decreasing the release of GLU.

Considering that CEMR is composed of different secondary metabolites, the activity of the aqueous (AFMR) and methanolic (MFMR) fractions, on the neuromuscular junction of cockroaches were evaluated (Fig. 3B-C). In these trials, MFMR did not induce significant changes in muscle contraction strength when compared to control (Fig. 3B). In contrast, AFMR induced a significant reduction of contraction force (Fig. 3C), time-dependent effect, since a greater decrease in muscle contraction was observed at the end of 120 min ($75 \pm 3\%$) in relation to control ($101 \pm 1\%$) ($n=6$, $p < 0.05$).

To identify the compounds responsible for the modulation of muscle contraction force induced by EBMR, the AFMR and MFMR fractions were synergistically evaluated in CNP. The results suggest that AFMR is the main compound involved in the neuromuscular blocking response of *M. rufula* since the concentration of 40 µg/g of animal was more effective when compared to 50 µg/g for animal of CEMR (Fig. 3D). The combined administration of the two fractions did not result in a synergistic effect on the AFMR alone, corroborating the previous data, which indicate the AFMR as responsible for the effect of the CEMR.

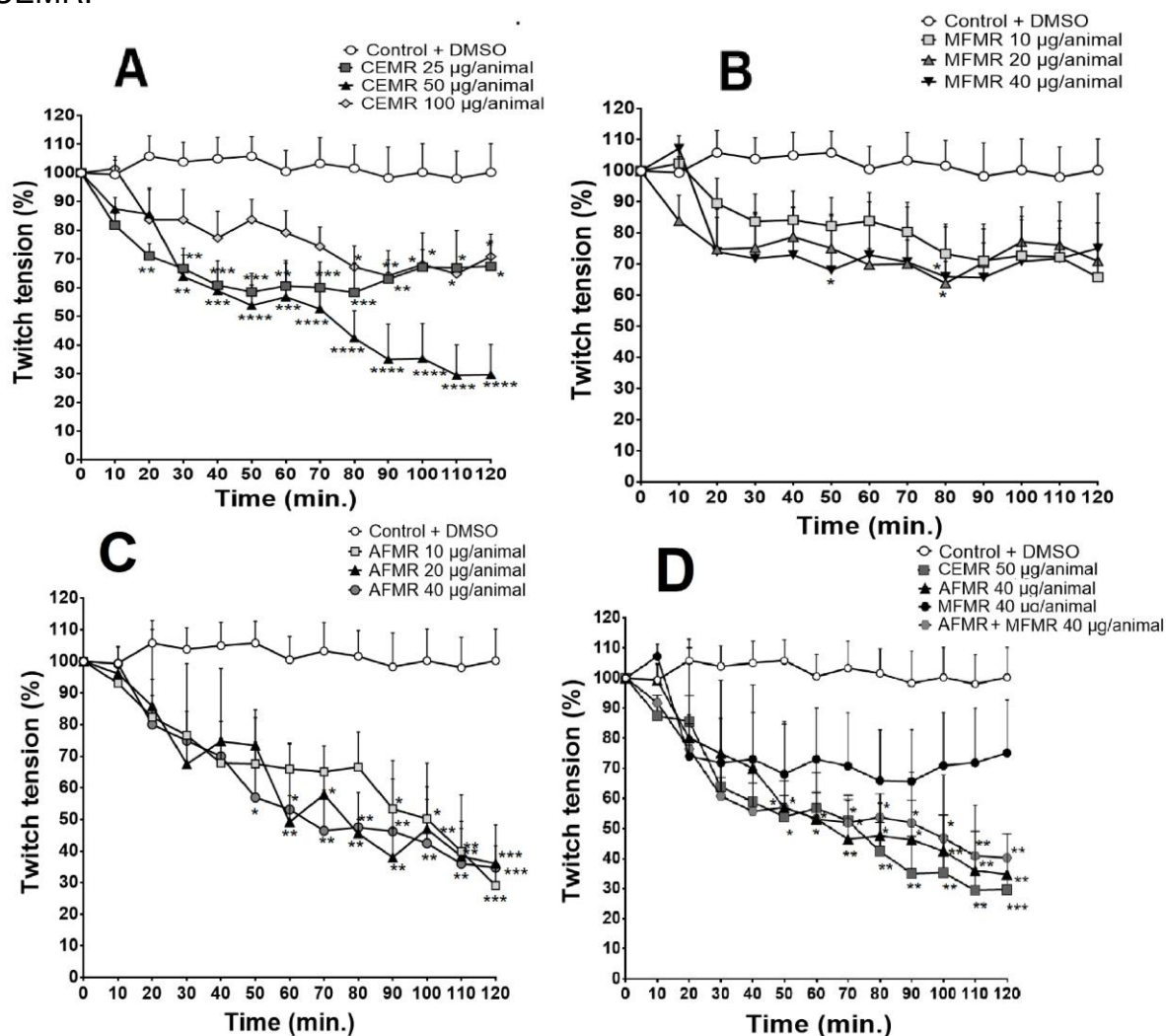


Figure 3: Effect of CEMR and fractions of *Manilkara rufula* (AFMR and MFMR) on the muscle contraction force of cockroaches of the species *Nauphoeta cinerea*. The author (2017).

4. FINAL CONSIDERATIONS

The results obtained in this study demonstrate that both *M. rufula* extract and its isolated compounds exert entomotoxic activity. This activity was characterized by the induction of a state of lethargy in the animals, reduction of the *grooming* rate and reduction of muscle contraction force. It has been demonstrated that the compounds present in the aqueous fraction are the main involved in this modulation. The results also show that the octopaminergic pathway is being modulated by CEMR and its

fractions, inducing an antagonistic effect on this pathway, evidencing the biotechnological potential of this plant.

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