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EVALUATION OF BARU (DIPTERYX ALATA) FLOUR AS A POTENTIAL INHIBITOR OF THE LACHESIS MUTA MUTA SNAKE VENOM -INDUCED MYOTOXICITY AND NEUROMUSCULAR BLOCKADE

AVALIAÇÃO DA FARINHA DE BARU (DIPTERYX ALATA) COMO POTENCIAL INIBIDOR DA MIOTOXICIDADE E BLOQUEIO NEUROMUSCULAR INDUZIDOS PELO VENENO DE COBRA LACHESIS MUTA MUTA

EVALUACIÓN DE LA HARINA DE BARU (DIPTERYX ALATA) COMO POTENCIAL INHIBIDOR DE LA MIOTOXICIDAD Y EL BLOQUEO NEUROMUSCULAR INDUCIDOS POR EL VENENO DE SERPIENTE LACHESIS MUTA MUTA

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ABSTRACT
Snake venom is a complex mixture of bioactive substances known to induce a wide range of toxic effects. We investigated the potential of baru flour (Dipteryx alata) in mitigating the neuromuscular blockade induced by Lachesis muta muta venom. Three pharmacological models (pre-incubation, post-venom, and pre-venom), with n=6 each, were exposed to isolated nerve-muscle preparations and conventional myographic technique. Muscular changes were further examined through light microscopy, and protein composition was analyzed via SDS-PAGE electrophoresis for both venom and baru:venom mixture. A concentration of 1000 µg/mL baru flour was chosen for subsequent assays against the venom (100 µg/mL), which inhibited 62.1 % of the contractile response. Notably, pre-incubation with baru flour resulted in a significant 30.6 % inhibition ($p<0.05$), while post-venom and pre-venom models showed no significant effects. Myotoxicity (in %) revealed that venom affected 35.3 ± 1.7 to cells, while pre-incubation,19.3 ± 1.9 ($p<0.05$). Electrophoresis analysis of the mixture containing baru flour and venom revealed a reduction in venom-specific proteins, a finding corroborated by a decrease in protein concentration (in µg/mL) from 6.8 ± 0.001 to 1.4 ± 0.003. Concluding, baru flour possesses significant potential as an inhibitor against paralysis and myotoxicity induced by Lachesis venom, a promising therapeutic application.

Keywords: Dipteryx alata Vogel. Lachesis muta muta. Neuromuscular junction.

RESUMO
O veneno de serpente é uma mistura complexa de substâncias bioativas conhecidas por induzir uma ampla gama de efeitos tóxicos. Investigamos o potencial da farinha de baru (Dipteryx alata) na mitigação do bloqueio neuromuscular induzido pelo veneno de Lachesis muta muta. Três modelos farmacológicos (pré-incubação, pós-veneno e pré-veneno), com n=6 cada, foram expostos a preparações nervo-músculo isoladas e técnica miográfica convencional. As alterações musculares foram ainda examinadas através de microscopia de luz e a composição proteica foi analisada através de eletroforese SDS-PAGE para ambos o veneno e a mistura baru:veneno. Uma concentração de 1000 µg/mL de farinha de baru foi escolhida para ensaios subsequentes contra o veneno (100 µg/mL), o que inibiu 62,1% da resposta contrátil. Notavelmente, a pré-incubação com farinha de baru resultou em uma inibição significativa de 30,6% ($p<0,05$), enquanto os modelos pós-veneno e pré-veneno não mostraram efeitos significativos. A miotoxicidade (em %) revelou que o veneno afetou 35,3 ± 1,7 células, enquanto a pré-incubação, 19,3 ± 1,9 ($p<0,05$). A análise de eletroforese da mistura contendo farinha de baru e veneno revelou uma redução nas proteínas específicas do veneno, um achado corroborado por uma diminuição da concentração de proteína (em µg/mL) de 6,8 ± 0,001 para 1,4 ± 0,003. Concluindo, a farinha de baru possui potencial significativo como inibidor da paralisia e miotoxicidade induzidas pelo veneno de Lachesis, uma aplicação terapêutica promissora.

Palavras-chave: Dipteryx alata Vogel. Junção Neuromuscular. Lachesis muta muta.

RESUMEN
El veneno de serpiente es una mezcla compleja de sustancias bioactivas que inducen una amplia gama de efectos tóxicos. Investigamos el potencial de la harina de barú (Dipteryx alata) para mitigar el bloqueo neuromuscular inducido por el veneno de Lachesis muta muta. Tres modelos farmacológicos (preincubación, posveneno y preveneno), con n=6 cada uno, se expusieron a preparaciones de nervio-músculo aisladas y a la técnica

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miográfica convencional. Los cambios musculares se examinaron con mayor detalle mediante microscopía óptica y la composición proteica se analizó mediante electroforesis SDS-PAGE tanto para el veneno como para la mezcla barú:veneno. Se seleccionó una concentración de 1000 µg/mL de harina de barú para ensayos posteriores contra el veneno (100 µg/mL), que inhibió el 62,1 % de la respuesta contráctil. Cabe destacar que la preincubación con harina de barú resultó en una inhibición significativa del 30,6 % ($p < 0,05$), mientras que los modelos pre y post-veneno no mostraron efectos significativos. La miotoxicidad (en %) reveló que el veneno afectó a 35,3 ± 1,7 células, mientras que la preincubación, a 19,3 ± 1,9 ($p < 0,05$). El análisis electroforesis de la mezcla que contenía harina de barú y veneno reveló una reducción en las proteínas específicas del veneno, hallazgo corroborado por una disminución en la concentración de proteínas (en µg/mL) de 6,8 ± 0,001 a 1,4 ± 0,003. En conclusión, la harina de barú posee un potencial significativo como inhibidor de la parálisis y la miotoxicidad inducidas por el veneno de Lachesis, una aplicación terapéutica prometedora.

Palabras clave: Dipteryx alata Vogel. Lachesis muta muta. Unión neuromuscular.

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INTRODUCTION

Snakebites, classified as neglected tropical disease by the World Health Organization (WHO), pose a significant global health concern, affecting annually 5.4 million people worldwide. Among these cases, between 81,000 and 138,000 result in fatalities, while up to 400,000 individuals experience permanent disability or disfigurement. These incidents predominantly affect impoverished populations residing in rural areas, resulting in various socio-economic consequences, including discrimination, abandonment, income loss, social divisions, mental health issues, and a reduced quality of life (Ministério da Saúde, 2019).

Between 2000 and 2015, Brazil recorded approximately 250,000 snakebite incidents, with around 100,000 cases classified as moderate to severe (Sinan, 2017). Due to the substantial frequency of these incidents, snakebites are included in Brazil's Compulsory Notification List (CNL), as stipulated by Ordinance No. 204, dated February 17, 2016. In Brazilian territory, medically significant snakes belong to various genera, including *Bothrops* (pit vipers), *Crotalus* (rattlesnakes), *Lachesis* (surucucus) in the family Viperidade, *Micrurus* (true corals) in the family Elapidae, as well as species of green snakes in the *Philodryas* genus, and musurans in the *Clelia* and *Boiruna* genera, belonging to the family Dipsadidae (Soares, 2012).

The snake *Lachesis muta* L. (1766), commonly known as "surucucu", holds the distinction of being the largest venomous snake in the Americas, with a maximum length of 3.5 meters [4]. Its presence in Brazil is predominantly documented in preserved areas and their vicinity, where native forests remain well-conserved (Funasa, 2001; Melgarejo, 2009; Campbell, 2004). This terrestrial and nocturnal species primarily preys of small to medium-sized mammals (Melgarejo, 2009). Its geographical range encompasses Panama in Central America and several South American countries, including Brazil, where it inhabits the Atlantic Forest biome, the Amazon region, and northern Mato Grosso (Almeida et al, 2007).

According to data from the Notifiable Diseases Information System (SINAN) website, a Ministry of Health platform containing valuable health indicators, there were over 7,000 *Lachesis muta* snakebite incidents recorded between 2007 and 2015, resulting in approximately 50 deaths (Sinan, 2017). Victims of *Lachesis* envenomation typically experience intense and throbbing pain at the bite site, along with rapid edema and inflammation within minutes. Additionally, symptoms may include bleeding disorders, nausea, vomiting, abdominal cramps, diarrhea, kidney dysfunction, profuse sweating,

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hypotension, bradycardia, and shock (Fernandes; Franco; Fernandes, 2004; Jorge et al, 1997; Pardal et al, 2007). Its venom exhibits potent proteolytic activity, disrupting blood clotting mechanisms, leading to bleeding; besides, it shares similarities with bothropic toxins but differs in its effects, manifesting as symptoms such as diarrhea, bradycardia, arterial hypotension, and shock (Souza, 2009; Diniz; Oliveira, 1992).

Amazonas, being a vast state, presents considerable challenges in terms of transportation, with many remote areas far from service centers. However, a significant portion of its population is concentrated in urban regions, such as Manaus and Parintins (Dantas; Silva; Silva, 2022). This urban concentration requires individuals to travel a substantial distance to access antivenom treatment. In contrast, the situation in the state of Maranhão differs, with a more dispersed population across the territory. Here, a significant proportion of the population resides far from service centers, leading to a situation where nearly 30% of the local population is more than two hours away from medical assistance. This distribution challenge raises concerns about providing timely aid to snakebite victims.

The primary treatment for snakebite envenomation involves the administration of specific antivenoms tailored to the type of poisoning. These specific antivenoms are the only effective treatment and should be administered in a hospital setting under medical supervision (Secretaria da Saúde, 2021). In cases of envenomation by *Lachesis muta*, the specific antivenom is known as antilachetic serum or antibothropic-lachetic serum, which should be administered intravenously. However, in situations where the availability of specific sera is limited, treatment can be initiated with antibothropic serum. It is important to note that while antibothropic serum may offer some benefits, it may not effectively neutralize the coagulant effects of *Lachesis* venom (Ministério da Saúde, 2005).

The potential use of plants or plant-derived compounds as antivenoms or as adjunct therapies for snakebites is an area of ongoing research. Some plants may contain bioactive compounds that could have a role in mitigating the effects of snake venom, but this research is in its early stages, and the effectiveness and safety of such treatments have yet to be established through rigorous scientific studies. *Dipteryx alata* Vogel, a member of the Leguminosae-Mimosoideae (Fabaceae) family, has long been recognized for its medicinal properties (Dos Santos; Lolis; Dal Belo, 2006). Its fruits are consumed by both animals (Lorenzi, 1992) and humans (Togashi & Sgarbieri, 1995). This plant, commonly known as “baru” or “cumaru” have molecules able to mitigate the neuromuscular blockade induced by *Bothrops jararacussu* venom, such as betulin, phenolic acid, flavonoids, isoflavone, and

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tannins; but these molecules did not protect against the *Crotalus durissus terrificus* venom (Nazato et al, 2010; Ferraz et al, 2012; Ferraz et al, 2014; Ferraz et al, 2015).

Nutritionally, the fruit of *D. alata* is a rich source of carbohydrates, proteins, lipids, and essential minerals. Furthermore, it contains noteworthy levels of bioactive compounds, including polyphenols and carotenoids (Lima et al, 2022). Considering its promising nutritional composition, numerous studies are investigating the potential health benefits of baru flour. The study aims to investigate the concentration-dependent effects of baru flour on preserving muscle-function and reducing venom-induced neuromuscular and myotoxic effects of *Lachesis muta muta* venom.

MATERIAL AND METHODS

BARU FLOUR

Baru flour is marketed as Baruzeiro, Baru chocolate, baru fruit pulp, extracted from *Dipteryx alata* by Agronomist Cesar Augusto Sandri, Babilônia farm (Mineiros, Goiás, Brazil). Comprises 65.01 ± 0.19 of carbohydrates; 3.30 ± 0.26 of lipids; 4.45 ± 0.06 of proteins; 1.79 % ± 0.01 of ashes; and 4.39 ± 0.16 of crude fibers. Baru flour was prepared following the steps: sieving (48 Mesh); weighing the desired concentrations for testing; solubilization in 5 mL Tyrode solution; and addition into the bath containing the experimental preparation.

LACHESIS MUTA MUTA VENOM

The venom of *L. m. muta* was provided by Professor Dr. Rafael S. Floriano, University of West Paulista, SP, Brazil; collected and certified by Dr. Nelson J. da Silva Jr. from the Center for Biological Studies and Research at the Catholic University of Goiás (Goiânia, GO). The venom was supplied in lyophilized form, ensuring its stability during storage and solutions were prepared in Tyrode's nutrient solution at the time of use.

ANIMALS

Male mice of the Swiss lineage, weighing between 25-30 g ensuring uniformity in the experimental group, were purchased from Anilab Animais para Laboratório (Paulínia, SP, Brazil), and placed in cages in the vivarium at Apoio 2 of the University of Sorocaba. Four mice/cage to ensure their well-being and comfort were placed with exhaustion and appropriate ventilation (environmental microventilation system, Smaflex®), with light/dark

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cycles every 12 hours controlled by a timer. The animals were accommodated on shaving beds free of chemical substances and received food and water *ad libitum*. The project was submitted to the Committee on Ethics in Animal Use - CEUA of the University of Sorocaba - SP, with protocol number 192/2020, approved on 10/08/2020.

PHRENIC NERVE-DIAPHRAGM PREPARATION

To start the protocols, the mice are anesthetized with Isoflurane by inhalation (100 mg/mL, BioChimico, Itatiaia, RH, Brazil), and subsequently exsanguinated by sectioning and bleeding the cervical vessels. The phrenic nerve-diaphragm preparation is isolated according to Bülbring (1997) (Bülbring, 1997), modified for mice. The preparation is removed and accommodated in a vat with a capacity of 5 mL, containing Tyrode's solution (nutritive solution: pH: 7.0, with the following composition (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 0.49; NaH_2PO_4 0.42; $NaHCO_3$ 11.9; and glucose 11.1), through the rib muscles by hooks at the base of the vat. The preparations are maintained at a temperature of 37 ºC, in a water bath, aerated with carbogen (mixture of 95 % O_2 and 5 % CO_2). A tension of 5 g/cm is applied through a wire attached to the tendinous portion of the muscle and attached to the transducer. The phrenic nerve is superimposed on an electrode that remains in contact with the surface of the nutrient solution. The force of contraction is measured by an isometric transducer (cat. 7003, Ugo Basile, Italy) and recorded by the digital system (Data Capsule, cat. 17.400, Ugo Basile) containing a basic pre-amplifier (cat. 7080, Ugo Basile), coupled to a computer via a USB interface for data storage. After that, the preparations are stimulated through the phrenic nerve, with supramaximal stimuli and a frequency of 0.1 Hertz lasting 0.2 ms provided from a double physiological stimulator (model ESF-15D, Ribeirão Preto, SP, Brazil). After recording under controlled conditions for 10 min of preparation stabilization, concentration- response curve for selecting the concentration of baru flour (200, 500 and 1000 μg/mL) and pharmacological models were carried out: pre-incubation (previous mixture during 30 min before adding into the bath of 100 μg/mL venom + 1000 μg/mL baru flour), pre-venom (pre-treatment of the preparation with baru flour for 10 min followed by adding the venom), and post-venom (venom addition during 10 min followed by adding the baru flour).

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HISTOLOGICAL ANALYSIS

The best outcome from pharmacological assay against the neuromuscular blockade induced by *L. m. muta* venom was qualitatively and quantitatively analyzed by light microscopy and compared with Tyrode controls, venom and baru flour. For this, three preparations were selected and fixed in 10 % formalin solution and processed through routine histological methods. Sections of 5 µm of the preparations were stained with hematoxylin-eosin 0.5% (weight/volume) for further microscopic analysis and quantification of damage (edema, myofibril condensation, ghost/myonecrosis and delta lesion) and compared to patterns of normal cells presenting an integral polygonal structure and a peripheral nucleus. For photographic documentation, the Zeiss AXIOSTAR Plus photomicroscope (Oberkochen, Germany) was used. The photos were taken at a magnification of 400x (40x objective, where a 1 cm bar = 40 µm) using an Iphone 7 digital camera with 12 megapixels and Samsung A71 with 64 megapixels of resolution. The counts (triple blind) of the cells were performed by 3 examiners, following established criteria where each person took 72 readings, with a total of 216 readings of 72 slides, divided between the pharmacological groups: baru flour (1000 µg/mL), Tyrode control, *L. m. muta* venom (100 µg/mL) and pre-incubation. Cell damage was expressed as myotoxicity index (MI), i.e., the percentage of the number of damaged cells divided by the total number of cells in three non-overlapping and non-adjacent areas of each preparation (Ferraz et al, 2014).

ELECTROPHORESIS SDS-PAGE

The assay was carried out according to the methodology described by Laemmli (1970), in which the protein profile of the venom analyzed in polyacrylamide gel electrophoresis (SDS-PAGE) (Gordon, 1995; Fagliari et al, 1998; Saquetti et al, 2008). A concentration of 4% was used in the application gel and 10% in the separation gel (100V; 30 mA; 10W). The venom (0.2 µg), flour (0.2 µg) and pre-incubation (venom - 0.2 µg + baru flour 0.2 µg) samples were applied in the presence of β-mercaptoethanol and, in parallel, a molecular mass standard from 10 to 250 kD, Dual Color Precision Plus (Bio Rad), was used. It was used for staining the Comassie-Bio Rad electrophoresis gel. After the run, the gel was washed in running water for 5 min, repeating the same procedure 3 times. The water was removed, and the Comassie-Bio Rad colors added, 50 mL per gel. The gel was

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gently shaken for 1 hour. The gel was washed for 30 minutes with water. Gels were stored in water.

PROTEIN DOSAGE

The standard curve was developed with the Protein Albumin from the Dosage Kit monoreagent total proteins, colorimetric test, Bioclin (Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil). Its initial concentration was 4 g/dL, and it was necessary to adjust its concentration to reach the requested protein concentration for carrying out the Bradford method (1976) (Bradford, 1976) of protein dosage. For that, 50 µL of the protein in known concentration (Albumin 4 g/dL) was diluted in 950 µL of Tyrode (nutritive solution used to preserve the viability of the isolated preparations and venom solubilization) in a 1.5 mL vial at room temperature environment, thus reaching the desired concentration of 2 mg/mL. From this moment on as dilutions necessary for making the concentration curve were started, following as literature recommendations, just doubling the volumes (maintaining concentration). After the contact of protein with 1 mL of Bradford reagent (Sigma-Aldrich) remaining at rest for 5 minutes controlled by a timer, the absorbance of each tube, in triplicate, was taken at 595 nm in the MultiSpec-1501 UV-VIS spectrophotometer Shimadzu Spectrophotometer.

STATISTICAL ANALYSIS

Results were expressed as mean + standard error of mean (SEM). The significance of the observed differences was determined by the One-way Anova followed by Tukey test, with p value < 0.05, using the statistical software Origin 9.5 (OriginLab Corporation, Northampton, MA, USA).

RESULTS

Baru flour was prepared for experimentation by initially sieving it through a 48 Mesh sieve. It was weighed at different dose concentrations of 200, 500 and 1000 µg/mL and solubilized in Tyrode (5 mL) before being added to the preparation (n=6, each). Figure 1 illustrates the results obtained in mean ± standard error of the mean (SEM) over a 2- hour experimental period, with measurements taken every 10 minutes.

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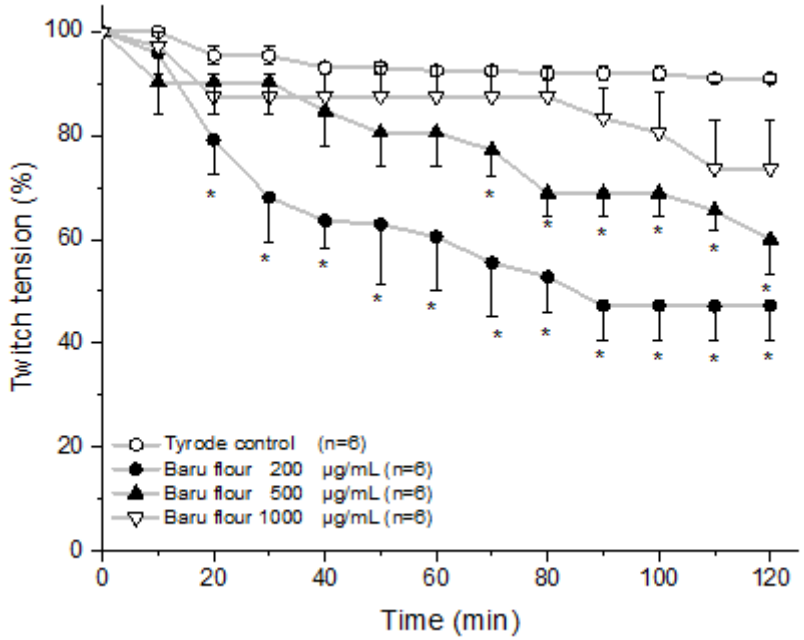
Figure 1. Mouse phrenic nerve-diaphragm preparation, indirect stimuli. Baru flour concentration-response curve was generated by testing 3 concentrations (in µg/mL): 200, 500 and 1000. Each data point on the curve represents the means ± standard error of the mean (SEM). The number of experiments (n) is indicated in the figure caption. *, p<0.05 compared to the Tyrode control.



It is noteworthy that the concentration of 1000 µg/mL exhibited an initial decrease in contractile response within the first 20 min of the experiment. However, this response stabilized and was maintained for up to 80 min before gradually decreasing until the end of the end 120 min experiment. Notably, this concentration demonstrated the highest degree of contractile response preservation, with no statistically significant difference observed compared to the Tyrode control. For this reason, the concentration of 1000 µg/mL was selected for the following neutralization studies.

Figure 2 shows the pharmacological models applied of (A), pre-incubation (venom + baru flour), (B), pre-venom (baru flour followed by the venom), and (C), post-venom (venom followed by the baru flour). First, notice that the *L. m. muta* venom at a concentration of 100 µg/mL exhibited a gradual decay over the 120 min experimental duration, resulting in a blockage of the curve. At the conclusion of the experiment, only 37.9 % ± 6.3 of the muscle remained responsive (*, p<0.05 compared to the Tyrode control).

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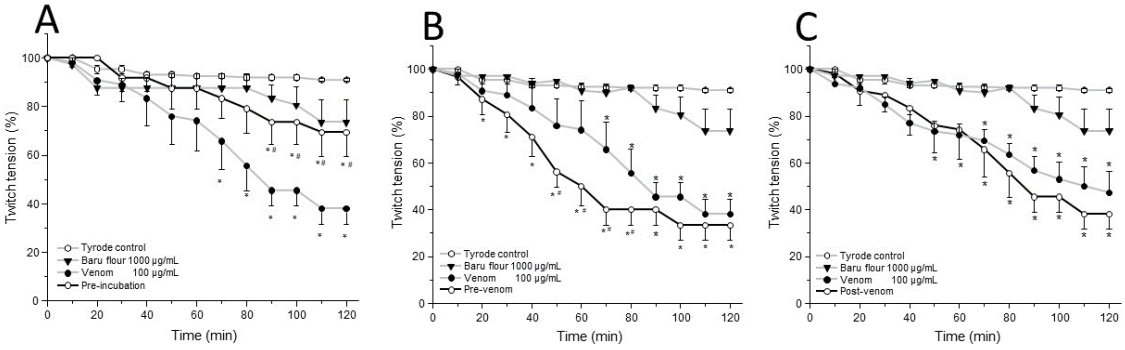
Figure 2. Mouse phrenic nerve-diaphragm preparation, indirect stimuli. Pharmacological models. A, Pre-incubation. B, Pre-venom. C, Post-venom. Each data point on the curve represents the means ± standard error of the mean (SEM). The number of experiments was of n=6 for each protocol. *, p<0.05 compared to the Tyrode control. #, p<0.05 compared to the venom.



To assess the potential neutralization effect (A), a mixture containing *L. m. muta* venom (100 µg/mL) and baru flour (1000 µg/mL) was pre-incubated for 30 min, protected from light. Subsequently, this mixture was added to the preparation for analysis. Notice the protection exerted by the baru fruit flour, which maintained 69.4 % ± 10 of functioning fibers. This finding underscores the potential of baru flour in mitigating the deleterious effects of the venom.

In the pre-venom pharmacological model (B), only 33.3 % ± 6.1 of muscle fibers remained responsive at the end of the experiment. Similarly, in the post-venom pharmacological model (C), only 47.2 % ± 9.1 were responsive at the end of the 120 min experiment. Significance levels are indicated in the figures as follows: *, p<0.05 compared to the control; #, p<0.05 compared to the venom. However, both pre-venom and post-venom pharmacological models failed to protect against the neuromuscular blockade induced by the venom.

To aid in our interpretation of the results, we assessed contractile efficiency (depicted in Fig. 3).

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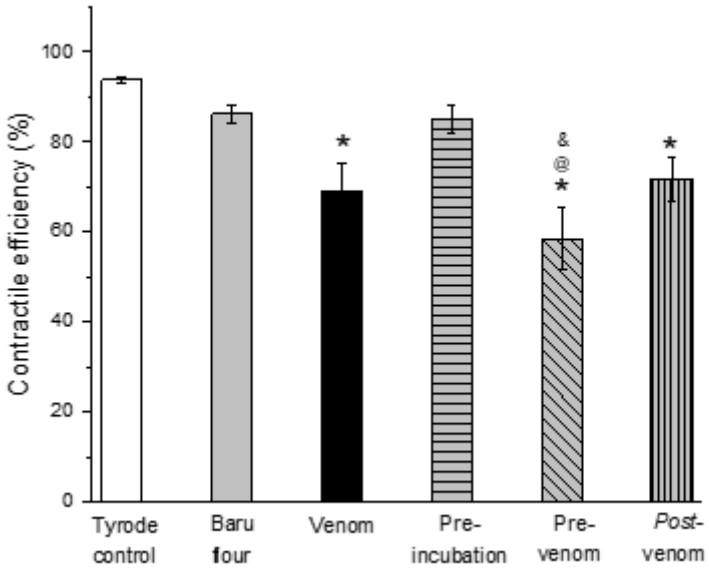
Figure 3. Mouse phrenic nerve-diaphragm preparation, indirect stimuli. Contractile efficiency (in %). The results show the resistance of the nerve-muscle machinery under the same conditions (experimental time, frequency, and duration) face to the different treatments. Notice that pre-incubation model has no statistical difference from Tyrode control and baru flour.



This methodology is particularly applicable to contraction protocols involving cyclic alterations in muscle length and force output remains consistent throughout the measurement period. Consequently, we examined contractile efficiency over the entire 120 min experimental duration. The muscular work (in %) yielded the following results: Tyrode control 93 ± 0.7; baru flour (specific intervention) 86.2 ± 2.0; venom treatment 69.1 ± 6.3; pre-incubation model 85.1 ± 3.1; pre-venom model 58.5 ± 7.0; and *post*-venom model 71.7 ± 4.8. This analytical approach offers valuable insights into the muscle's ability to generate work under varying conditions. It provides a perspective on how different factors, including treatments and interventions, influence the muscle's contractile performance.

Figure 4 presents a qualitative assessment using inserted photographs and a quantitative analysis of morphological changes observed under different treatment conditions, as indicated in the legend. The venom damaged 35.3 % ± 1.7; the baru flour, 21.6 % ± 1.7; Tyrode control, 13.8 % ± 3.5; and pre-incubation, 19.3 % ± 1.9 of muscle fibers at the end of the 120 min.

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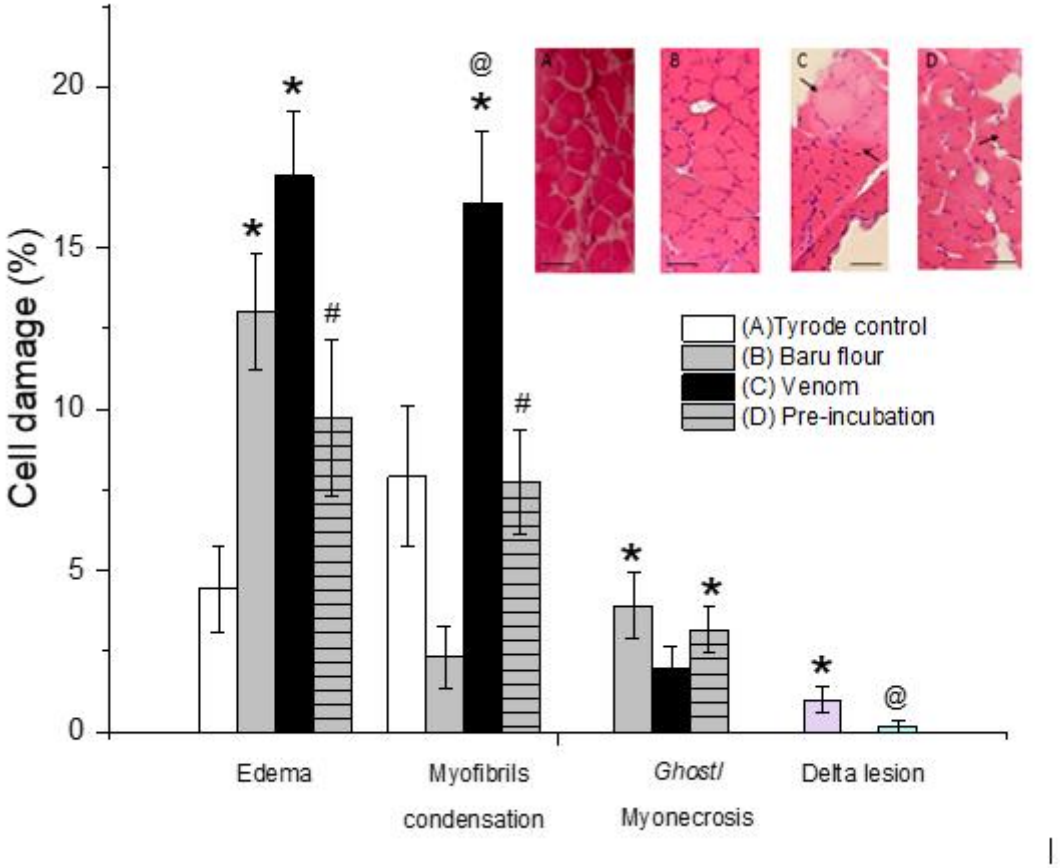
Figure 4. Qualitative (inserted photographs, Bar = 1 cm = 40 µm, H.E. stain) and quantitative morphological changes, according to the treatments shown in the legend of the figure. The analyzed morphological scores include: edema (highlighted by arrows in photograph C), myofibrils condensation, ghost and myonecrosis, delta lesion. Notably, a substantial edema is visible in photograph C, and there is a significant reduction observed in the pre-incubation model, as indicated by the arrow in the photograph D. p<0.05: *, compared to Tyrode control; #, compared to venom; @, compared to baru flour.



The results provide valuable insights into the qualitative and quantitative changes in morphology under various treatment conditions, highlighting the impact of different interventions on cellular features. However, it is important to note that none of the treatments completely prevent cellular damage, as evidenced by the presence of ghost/myonecrosis and delta lesion.

Aiming to confirm the venom attenuation in the pre-incubation model a protein electrophoresis was carried on SDS-PAGE and protein determination (in triplicate), as well. Figure 5 depicts protein with four distinct applications: 1, baru flour; 2, *L. m. muta* venom; 3, pre-incubation; 4, molecular mass markers (kDa).

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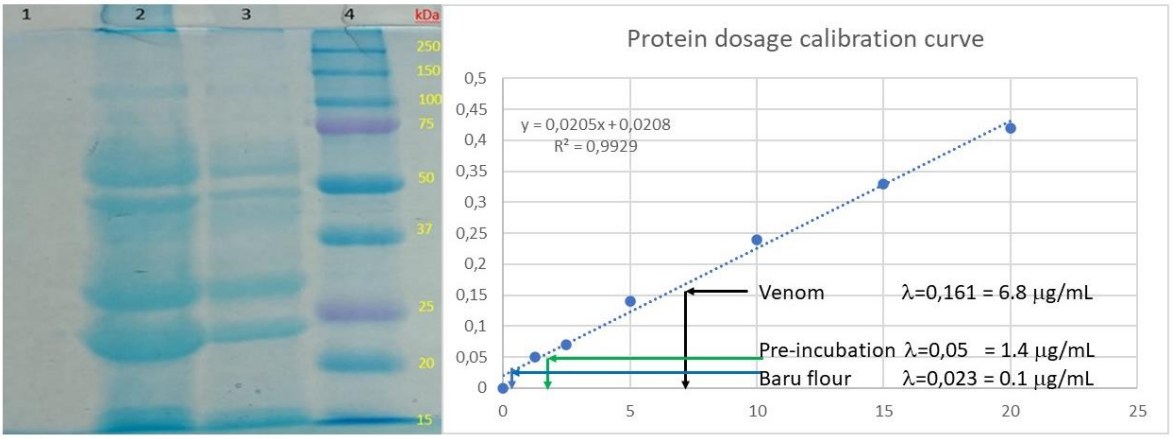
Figure 5. SDS-PAGE (10% polyacrylamide gel) in the presence of β-mercaptoethanol (0.5% v/v). The gel was stained with Coomassie Brilliant Blue G-250. kDa, molecular mass markers (kDa). 1, 2, 3 and 4 (see the text). The visual decrease observed in lane 3 (pre-incubation) compared to the venom alone (lane 2) is further quantitatively confirmed by protein determination. Each 10 μg/mL of venom has 6.8 μg/mL of protein, while baru flour = 0.1 μg/mL (see lane 1 empty) and pre-incubation = 1.4 μg/mL.



These results clearly show the protective effect of baru flour by electrophoresis profile as by protein determination. The mechanism by which the baru flour did it remains to be clear, but it is attractive to think in absorption (Guimarães et al, 2012).

DISCUSSION

The search for alternatives to mitigate the severity of snakebites is expanding. Simultaneously, there is a growing interest in harnessing the biotechnological potential of snake venom for the treatment of other diseases and cosmetic industry, as well (Soares, 2012). Here, the baru flour from *Dipteryx alata* with a particular focus on its potential to mitigate the neuromuscular blockade and myotoxicity induced by *L. m. muta*, was conducted. Then, the concentration-response curve was a fundamental step for guiding this study, offering insights into how the compound interacts with the biological system (Tavares, 2004). Interestingly, our findings revealed a noteworthy phenomenon where smaller concentrations of baru flour appeared to induce a greater blockage of the contractile response when compared to larger concentrations. While this may seem counterintuitive at first glance, it is not an isolated occurrence in pharmacological studies, using natural compounds. For instance, the hydroalcoholic extract of *Vellozia flavicans*, colloquially known as “canela-de-ema” exhibited a similar pattern (Tribuiani et al, 2014). The mechanism to elucidate the intricate interplay between endogenous signaling pathways (Malomouzh et al, 2003; Rubem-Mauro et al, 2009) and cholinergic control of muscle movement remains a subject of investigation.

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In the pre-incubation assay, but not in pre-venom and *post*-venom, baru fruit flour demonstrated a significant protective effect against the neuromuscular blocking induced by *L. m. muta* venom (#, $p<0.05$). This model indicates a direct interaction between the components of baru flour and the venom, occurring during the 30 min pre-incubation period. The use of pre-incubation has been validated as a valuable screening tool in the study of medicinal plants with antiophidic properties, as seem with *Coutarea hexandra* stem bark's aqueous extract for the management of acute systemic poisoning induced by *L. m. muta* in mice (Leão Torres et al, 2017), and *Galactia glaucescens* against neurotoxic and myotoxic effects of *Bothrops jararacussu* venom (Dos Santos et al, 2017), among others found in the literature

The possibility of an interaction between the carbohydrates found in baru flour and the proteins in *L. m. muta* venom is a plausible hypothesis, particularly given that carbohydrates constitute the predominant component, comprising approximately 65% of the flour's composition. One of the methods employed to explore this interaction is nuclear magnetic resonance spectroscopy (NMR spectroscopy) (Pérez; Tvaroška, 2014). Moreover, computer modeling techniques, such as docking simulations have been instrumental in elucidating potential interactions between these components such as - solvation effects, hydrogen bonding, van der Waals forces, and electrostatic interactions - to predict and visualize potential binding sites and mechanisms.

Electrophoresis techniques have also played a crucial role in elucidating the interaction between carbohydrates and venom proteins. Techniques such as capillary electrophoresis, PAGE (Analysis of Polysaccarides using Carbohydrates Gel Electrophoresis) (Goubet; Dupree; Johansen, 2011), and FACE (Fluorescence-Assisted Carbohydrate Gel Electrophoresis) (Robb et al, 2017) have been employed to assess changes in molecular profiles and to provide direct evidence of the interaction's impact on protein quantities. Furthermore, in our laboratory, we have access to a range of well-established resources for the analysis of snake venoms (Oliveira et al, 2020; Damico et al, 2006). Through electrophoresis experiments, we have observed a clear reduction in the quantity of proteins following the interaction between baru flour and venom. On the other hand, the electrophoresis profile for newborn and juvenile venoms of other species/subspecies of the genus *Lachesis* the venom protein bands is (Ripa, 2007): above 97 kDa equal to a metalloprotease; ranges from 33 to 53 kDa equal to a serine protease; ranges from 26 to 29 kDa equal to a metalloprotease; and ranging from 15 to 17 kDa equal

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to a PLA_2. To gain deeper insights into this phenomenon, we propose conducting molecular assays to quantify venom proteins and the resulting supernatant from the pre-incubation process. This approach will allow us to quantitatively assess the extent of protein reduction and provide a more comprehensive understanding of the interaction dynamics. Combining electrophoresis and quantitative protein assays we confirmed the potential of baru flour to minimize the toxic effects of constituents from the venom.

The neurotoxicity (only 37.9% ± 6.3 of responsive cells) of *L. m. muta* are inversely related to myotoxicity (only 35.3 % ± 1.7 of damaged cells). The cytotoxic effects induced by *L. m. muta* venom can be attributed to the venom proteins' remarkable ability to provoke severe hemorrhage, resulting in cellular damage that, in turn, may lead to tissue necrosis (Alves, 2010). Furthermore, the observed cytotoxicity might be linked to the concentrations of specific venom components, such as phospholipase A_2 (PLA_2), which account for 8.7 % of the venom's composition. These enzymes play a crucial role in the disruption of cell membrane, causing cellular damage and contributing to the cytotoxic effects observed (Oliveira et al, 2021). Besides, they coexist with other venom components, including metalloproteinases and serine proteinases, which make up 31.9 % and 31.2 % of the venom composition, respectively. These diverse venom constituents collectively contribute to the venom's multifaceted effects on cellular and tissue integrity.

CONCLUSION

Baru flour significantly protected against the blocking effect of *Lachesis muta muta* venom, in the pre-incubation model. The hypothesis of the flour:venom interaction may be due to the flour carbohydrates absorbing the venom proteins, which still needs to be confirmed.

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CONFLICTS OF INTEREST

Not applicable.

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