

Antinociceptive effects of saccharose and aqueous extract of *Cordyline dracaenoides kunth* (uvarana) in experimental models after induction of hyperalgesia using capsaicin

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OBJECTIVE: There is evidence that sweet substances such as saccharose can enhance the analgesic properties of endogenous opioids, leading to pain relief; it is also known that *Cordyline dracaenoides Kunth*, commonly known as uvarana, is used in folk medicine as an anti-inflammatory and analgesic agent. The aim of the present study was to compare the antinociceptive effects of uvarana aqueous extracts vs. saccharose in rats.

METHOD: Twenty-four Wistar rats were used, divided into two groups of twelve, namely a uvarana and a saccharose group. Capsaicin was used to induce hyperalgesia and the nociceptive threshold was assessed every five minutes for a total of 50 minutes. Baseline values were obtained and this was followed by administration of uvarana or saccharose at three different concentrations (100, 250 and 300 g/L). The nociceptive threshold was assessed using the tail flick test.

RESULT: In comparison to baseline values, uvarana and saccharose provoked significant and comparable antinociceptive effects at concentrations of 250 g/L and 300 g/L, respectively.

CONCLUSION: Both substances caused similar antinociceptive effects in comparison to baseline values.

KEYWORDS: analgesia; saccharose; uvarana.

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INTRODUCTION

According to the International Association for Study of Pain, pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage, which is experienced by everybody.¹ Nociception is associated with recognizing signs of pain through the nervous system, which prepares information related to this damage² and is defined as the physiological component of pain, which consists of three processes of neural signals generated in response to an external stimulus: transduction; transmission and modulation.³ Consequently, antinociception can be defined as a decrease in the response of the sensory systems to the stimulus of pain.⁴

There is evidence indicating that the consumption of sweet substances increases the activity of the endogenous opioid peptides in the nervous system of animals,⁵ and in human plasma.⁶ Thus, such substances may interact with

the endogenous opioid system, affecting sensitivity levels.⁷ This association has led many researchers to examine the existence of a possible connection between this intake and antinociception. A number of studies have shown that the ingestion of sweet solutions increases the latency of the paw withdrawal response during the tail flick test.⁸⁻¹⁰

Cordyline dracaenoides Kunth, commonly known as uvarana, belongs to the Agavaceae family. It is the only neotropical species of the *Cordyline* genus and is a monocot plant the size of a small tree (up to nine meters in height).¹¹ The species is used in folk medicine as an anti-inflammatory, an analgesic and to treat rheumatism and associated diseases.¹²

Saccharose (common table sugar) is the most common natural disaccharide. It is composed of D-glucose and D-fructose¹³ and used as a non-pharmaceutical intervention that is efficient in terms of pain relief in children and neonates.¹⁴

Capsaicin is a compound of red peppers that can affect pain, inducing hyperalgesia or inflammation of C-type fibers.¹⁵ Dimethylsulfoxide (DMSO) is a chemical organic compound,¹⁵ which is capable of inducing cellular fusion

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and differentiation; it also improves the permeability of lipid membranes, thereby favoring the penetration of substances through them.¹⁷ Capsaicin, when associated with DMSO, spreads more efficiently through the membranes allowing a faster induction in hyperalgesia, which was a requisite in the present study.

Algesimetric tests, such as the Tail-Flick Instrument, a device that measures the degree of analgesia arising from treatment that affects the perception of nociceptive stimuli, can produce antinociception evaluation through different systems in a single antinociceptive system.¹⁸

The aim of the present study was to assess the antinociception produced, in experimental models, by uvarana, in comparison to saccharose following the induction of hyperalgesia through a subcutaneous injection of capsaicin diluted in Dimethylsulfoxide.

MATERIALS AND METHODS

Animals

Twenty-four male Wistar rats (*Rattus Norvegicus*), with a body weight between 200 and 250 grams, were provided by the vivarium of UNICENTRO-PR. The animals were kept in pairs in acrylic cages, in a light/dark cycle of 12 hours and temperatures of $22 \pm 1^\circ\text{C}$, with free ad libitum access to food and water.

All of the tests were conducted following the standards of the National Commission on Animal Experimentation, and were approved by the Ethics Committee for Animal Use of the Midwestern State University under protocol number 033/2012.

Nociceptive tests

The nociceptive threshold of all of the animals was measured using the tail flick test. Each animal was placed in a containment cell with acrylic walls. The tail was then placed on the sensor of a heat source (Tail-Flick Instrument; Stoelting). The progressive heating of the instrument was immediately interrupted when the animal removed its tail from the device. Slight adjustments of intensity and current were performed in the beginning of each experiment, in order to obtain three consecutive basal tail withdrawal latencies (TWL) between 2.5 seconds and 3.5 seconds. When the animal did not remove its tail from the heat source within 6 seconds, the device was turned off to prevent tissue damage. Baseline values were determined as the mean of three tail withdrawal test values, taken at intervals of five minutes, before uvarana or saccharose administration.

Preparation of the extract

To prepare the aqueous extract of *Cordyline dracaenoides Kunth*, the root was first dehydrated in an oven at 37°C for approximately 72 hours and then ground in an electric mill. The next phase focused on the decoction of 200 g of uvarana root powder, diluted in 1 liter of water and boiled for 15 minutes. Next, 200 ml of the boiled substance was extracted and lyophilized. After lyophilization, the extract was weighed and diluted to 100 g/L, 250 g/L and 300 g/L in 1 liter of distilled water.

Experimental procedures

All procedures were carried out in the Laboratory of Anatomy and Neurophysiology, Department of Physiotherapy, Midwestern State University, Guarapuava, Paraná, Brazil.

All of the animals initially received a single dose of $50 \mu\text{l}$ of capsaicin (2%), diluted in DMSO, in the underside of the middle third of the tail. Animals were submitted to the tail-flick test to gauge their nociceptive thresholds.

The nociceptive threshold was also measured in sequence with the administration of saccharose solution or uvarana extract every five minutes for 50 minutes. This was based on evidence that hyperalgesia induced by capsaicin exhibits a mean duration of 90 minutes.^{19,20}

The animals were divided into two experimental groups: a group in which the aqueous extract of uvarana was ingested, sub-divided into UG100, UG250 and UG300; a group in which saccharose was ingested, sub-divided into SG100, SG250 and SG300. Each sub-division contained four animals. Baseline recordings were obtained for each group before the administration of uvarana or glucose. Both substances were administered orally, at a quantity of 0.5 mL every 5 minutes for 50 minutes, using three different concentrations: 100 g/L; 250 g/L and 300 g/L.

Drugs

Commercial Saccharose (União[®]), Uvarana aqueous extract, capsaicin (Sigma[®]) and DMSO (Sigma[®]) were used.

Statistical Analysis

The GraphPad Prism 5.0 software was used, along with D'Agostino's test to confirm the normality of the samples. The Kruskal-Wallis test was used for inter-group analysis, with the significance level set at $p < 0.05$.

RESULTS

Figure 1 displays the mean values and standard deviations (in seconds) observed in the uvarana and saccharose groups for the tail withdrawal tests. The mean values and standard deviations in the uvarana group were as follows (in seconds): 4.42 ± 0.26 , 4.32 ± 0.43 , 5.34 ± 0.73 and 5.53 ± 0.67 and for baseline and the concentrations of 100 g/L, 250 g/L, and 300 g/L, respectively. For saccharose, mean values of 4.32 ± 0.43 , 5.43 ± 0.65 , 5.72 ± 0.70 and 5.59 ± 0.77 were found for baseline and the concentrations of 100 g/L, 250 g/L and 300 g/L, respectively.

Significantly higher mean values were found for the concentrations of 250 g/L and 300 g/L, which provided a greater increase in the nociceptive threshold of the animals. When the 250 g/L concentration was used, both substances

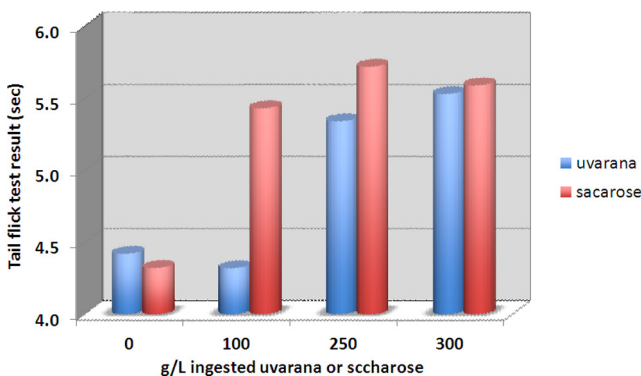


Figure 1 - Mean antinociception for uvarana extract and saccharose according to the different ingested concentrations.

Table 1 - Statistical significance vs. baseline

Group/Concentration	Statistical Value
Uvarana 100 g/L	$p = 0.808$
Uvarana 250 g/L	$p < 0.05$
Uvarana 300 g/L	$p < 0.001$
Saccharose 100 g/L	$p = 0.132$
Saccharose 250 g/L	$p < 0.001$
Saccharose 300 g/L	$p < 0.01$

resulted in mean values with an increased nociceptive threshold. When 250 or 300 g/L concentration was used, the uvarana group exhibited increases that were undistinguishable from those recorded in the saccharose group.

Table 1 displays the values found in the statistical analysis. In both groups, significant values were found in the concentrations of 250 g/L and 300 g/L.

DISCUSSION

The novelty in this study is the fact that uvarana extracts exhibited similar levels of antinociception in doses comparable to saccharose. To the best of our knowledge this is the first quantitative analysis of the antinociceptive effect of uvarana. We used saccharose as a term of comparison because these are well known previously reported results, as will be shown below.

According to Freitas et al.,²¹ acute oral administration of sweet substances, such as saccharose, to young and adult rodents induces a significant analgesic effect. The same authors expressed surprise at this finding since a number of previous studies, such as that by Segato et al.,²² suggested that only chronic ingestion of these substances would cause antinociception in adult mammals.

In the present study, a significant analgesic effect was observed as a result of acute administrations of uvarana solution, which was comparable to that of saccharose, particularly at the higher concentrations. All of the animals that orally ingested the higher doses of both substances exhibited significant and equivalent increases in their nociceptive threshold in both the acute and sub-acute phases (0-15 minutes).

Barr et al.²³ also found evidence that sweet substances in the mouth stimulate cortical areas associated with pleasure, causing physiological and sensory effects which liberate endogenous opioids, which in turn engage their own receptors, such as the μ receptor.

Segato et al.²⁴ investigated which concentration (62 g/L, 125 g/L e 250 g/L) of saccharose provided better antinociceptive results. Their results suggested that the most efficient concentration of saccharose was 250 g/L, which caused the most significant increase in the nociceptive threshold. These findings are corroborated in this study which shows, moreover, that raising saccharose concentration from 250 to 300 g/L does not lead to enhanced antinociception.

According to Calixto et al.²⁵ the brief analgesic effect of *Cordyline dracaenoides Kunth* is significant. This effect is caused by the presence of a complex of saponins, which are an important class of triterpenes in the terpenes group.

Beltrame et al.²⁶ recently noted that studies assessing the pharmacological properties of *Cordyline dracaenoides Kunth*, as well as its composition, are scarce. The same authors performed phytochemical screening using extract of the

plant and reported the presence of terpenes, including saponins and sterols. The presence of flavonoids was confirmed in the chemical composition of the plant under UV light (266 and 283 nm). In the present study, analysis of the aqueous extract from the root of the plant, including shaking, produced supernatant foam, which is characteristic of the presence of saponins.

A revision by Passos et al.²⁷ examined the properties of terpenes as members of a vast group of secondary metabolites, which act on the central nervous system, triggering sedative, anxiolytic and antinociceptive activities among others. They mainly act on the GABAergic, glutamatergic, dopaminergic and opioid neurotransmitter systems.

These statements strengthen the results obtained and stress the antinociceptive properties of the *Cordyline dracaenoides Kunth* when administered orally from aqueous extract, particularly in higher concentrations.

CONCLUSION

Cordyline dracaenoides Kunth exhibits an antinociceptive effect in its interaction with hyperalgesia induced by a subcutaneous injection of capsaicin diluted in DMSO, in experimental models. The effect is of the same magnitude as that produced by saccharose.

RESUMO

OBJETIVO: Há evidência de que substâncias doces, tais como a sacarose podem acentuar as propriedades analgésicas dos opióides endógenos, com alívio da dor; sabe-se também *Cordyline dracaenoides Kunt*, vulgarmente conhecida como uvarana, é utilizada na medicina popular como anti-inflamatório e agente analgésico. O objetivo do presente estudo foi comparar os efeitos antinociceptivos de extratos aquosos da uvarana com a sacarose em ratos.

MÉTODOS: Foram utilizados vinte e quatro ratos Wistar, divididos em dois grupos de doze, um tratado com uvarana e outro com sacarose. A capsaicina foi usada para a indução da hiperalgesia e o limiar nociceptivo foi avaliado a cada cinco minutos durante um total de 50 minutos. Valores basais foram obtidos e em seguida foram administradas oralmente extrato de uvarana ou sacarose em três diferentes concentrações (100, 250 e 300 g/L). O limiar nociceptivo foi avaliado através do teste de retirada da cauda (tail flick test).

RESULTADO: Em comparação com os valores basais, uvarana e a sacarose provocaram efeitos antinociceptivos significativos e comparáveis em concentrações de 250 e 300 g/L, respectivamente.

CONCLUSÃO: Ambas as substâncias causaram efeitos antinociceptivos semelhantes em relação aos valores basais.

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