

Resveratrol Prevents Motor Impairment Induced by Cassava Juice (*Manihot esculenta* Crantz) in Wistar Rats

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Abstract Cassava (*Manihot esculenta* Crantz) is a plant whose chronic consumption is associated with neurological diseases. On the other hand, resveratrol is a natural polyphenol that exerts antioxidant properties preventing the development of neurodegenerative diseases. However, it is unknown whether resveratrol can prevent motor incoordination induced by chronic consumption of cassava juice in rats. Fifty-six male Wistar rats were distributed in eight independent groups (n = 7 rats per group): Vehicle (water); Vehicle+Resveratrol; Cassava1+Vehicle; Cass2+Vehicle; Cass3+Vehicle; Cassava1+Resveratrol; Cassava2+Resveratrol; and Cassava3+Resveratrol. Treatments were orally co-administered every 24 hours for 28 consecutive days. One hour after the last administration, the effects were evaluated in the open-field, rota-rod, and swimming test. Cassava juice consumption increased motor activity in the open-field tests, induced spin behavior in the swimming test, and produced motor incoordination in the rota-rod test, which was prevented by the co-administration of resveratrol. The consumption of a dose of 10 mg/kg resveratrol for 28 days exerted a protective effect against motor impairment induced by cassava juice consumption in adult Wistar rats, probably associated with its antioxidant effects in the central nervous system.

Keywords: antioxidant, cassava juice, neurologic disease, motor impairment, resveratrol

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1. Introduction

Food consumption is considered one of the determining factors in the formation, development, and progress of society [1]. People commonly considering that food is safe, and that any danger always comes from processed products. However, this is not necessarily the case because even a natural food in good condition and that has not been modified or handled incorrectly can cause adverse effects on health. The naturally occurring toxicity in food products can be due to different factors such as content of potentially toxic substances that are generated during production or in the manufacturing processes [2]. Cassava (*Manihot esculenta* Crantz) is a major food ingredient and source of energy due to ease in production a storage, for which it constitutes the fourth most important staple diet worldwide after rice, wheat, and corn. It contains several glycosides which can cause potential toxic effects [3]. Many of “sweet varieties” have low levels of these glycosides and can be safely consumed after normal cooking processes. In contrast,

other “bitter varieties” have high levels of these glycosides that need an adequate processing so that they can be suitable for consumption [4]. Preclinical studies have consistently reported that the long-term consumption of cassava juice produces motor incoordination in rats at doses of 14.28 g/kg and 28.56 g/kg of cassava, which was found to be associated with cassava's content profile of neurotoxic glycosides [5,6]. On the other hand, resveratrol (3, 5, 4'- trihydroxystilbene) is a phytoalexin-type polyphenol found in some plants, predominantly in the exocarp of red grapes, red wine, some nuts, peanuts, and fruits such as berries [7]. Currently, there are numerous *in vitro*, *in vivo*, and epidemiological studies that suggest that resveratrol has beneficial effects on health. Some of the biological activities include anti-inflammatory, antiviral, antitumor, cardiovascular, and neuroprotective effects [8]. Likewise, one of the first described pharmacological properties of resveratrol is its antioxidant and protective activity on cell function, as it has inhibitory effects on oxidation agents, such as toxins and free radicals [9]. The mechanism by which it performs its protective effect has not yet been determined, but it has been proposed that it

activates SIRT1, an NAD⁺-dependent protein deacetylase that play an important role in cell survival by reducing oxidative stress and increasing mitochondrial biogenesis by deacetylating and activating transcription factors FOXO and PGC-1 α . SIRT1 has several important functions in the central nervous system, mainly in neuronal development and neuroprotection [8,10]. Therefore, in the present study, the potential protective effect of resveratrol was evaluated through prevention of motor alterations induced by cassava juice (*Manihot esculenta* Crantz) when co-administered in male Wistar rats. Hence it is hypothesized that the co-administration of cassava with resveratrol can prevent motor impairment in rats subjected to the open-field, swimming, and rota-rod tests.

2. Materials and Methods

2.1. Animals

A total of 56 male Wistar rats, two months old and weighing between 250 and 300 g at the beginning of the experiments, were used. Five rats per cage were housed in Plexiglas cages (33 x 44 cm base, 20 cm high) under a 12 h/12 h light/dark cycle (lights on at 7:00 a.m.) and at an average room temperature of 25°C $\pm$ 2°C. The animals had *ad libitum* access to water and food.

2.2. Ethical Approval

The experimental protocols were strictly performed according to the Guide for the Care and Use of Laboratory Animals [11] and Official Mexican Standard NOM-062-ZOO-1999 [12]; additionally, the recommendation stated by the 3 Rs of Russell (Reduce, Replace, and Refine), as applied to experimental research in animals, were considered [13]. The welfare of the rats was checked daily according to the rat grimace scale [14]. Body weights were recorded every three days. The protocol was approved by the Internal Committee for the Care and Use of Laboratory Animals of the Institute of Health Sciences (CICUAL-ICS), Universidad Veracruzana, with registration number 2018-002B, dated 18 February 2019.

2.3. Biological Material

All the cassava tubers (*M. esculenta* Crantz) used in the present study were collected from the same site and during the same season to minimize potential variation in their phytochemical profiles, as carried out in previous studies [6,15,16]. The cassava tubers were collected using traditional cultivation methods in the town of Colipa county, Veracruz state, México (latitude: 19°55'27" N; longitude: 96°43'48" W) at an altitude of 260 m above sea level. The authentication of the biological material was performed in the Herbarium XAL at the Institute of Ecology A.C. (INECOL) in Xalapa city by taxonomist Sergio Avendaño Reyes.

2.4. Cassava Juice

The extraction of cassava juice was performed according to previous studies in which the same cassava variety was

used, and identification and quantification of components were performed [15,16,17]. Every day, before administration, fresh cassava tuber juice was obtained with a juice extractor machine (Moulinex Model Centri III, Celaya, Guanajuato, México) and immediately administered to the rats according to the corresponding doses.

2.5. Dose Selection

To evaluate the effects of cassava juice (*Manihot esculenta* Crantz), different doses were used, taking into consideration previous studies [15], where the dose-response curve showed the effect of the administration of cassava juice on motor alterations. A dose of 7.14 g/kg was considered a medium level that does not produce motor deficit, while 14.28 g/kg represents the effective dose, which, according to the findings reported in previous studies of our study group, produces a motor deficit. A dose of 28.56 g/kg was considered a double dose that exacerbates motor disturbance.

2.6. Experimental Groups

A transversal study with eight independent groups (n = 7, rats/group) was performed: A vehicle group received purified water (Veh), three additional groups received the different doses of cassava juice (Cass1, 7.14 g/kg; Cass2, 14.28; and Cass3, 28.56 g/kg, respectively). Other three additional groups received the combinations of cassava juice plus resveratrol (RV) 10.7 mg/kg (Cass1+RV; Cass2+RV, and Cass3+RV, respectively). Resveratrol was purchased under the trade name of ResVitá@ (GNC Laboratories, capsules of 1.1 g each, containing 500 mg of resveratrol). For this research, products were used that have a pharmaceutical form and a standardized dose per unit; we did not use reagent grade active ingredients. Resveratrol was prepared and administered at 10.7 mg/kg, corresponding to two capsules per adult person, considering a weight of 70 kg. The capsules were dissolved in water, and, according to the weight of the rats, the calculation for the corresponding administration was made. The amount of water was established as 2 mL for the initial administration. However, this was increased in consideration of the weight of the rats. To carry out the administration of cassava juice and resveratrol, an intragastric cannula was used [18]. The animal was held in an upright position and the cannula was passed through the side of the mouth, between the incisors and premolars, into the esophagus, where the cassava juice and resveratrol were deposited. The experimental Cass1+RV, Cass2+RV, and Cass3+RV groups were initially administered with resveratrol orally, half an hour after the cassava juice extract has been administered to each rat, in the respective doses mentioned above. The administration of resveratrol and cassava juice to all the groups was performed every 24 hours (between 8:00 to 9:00 am).

2.7. Behavioral Tests

The rats were evaluated in a behavioral test battery that began with the open-field test, followed by the rota-rod test, and then the swim test. Approximately 2 to 3 min elapsed between tests.

2.7.1. Open-Field Test

The rats were individually placed in an opaque Plexiglas cage (44 cm × 33 cm base, 20 cm high). The floor was delineated into 12 squares (11 cm × 11 cm). In a first session (pretest, day 27 of treatment) the rats were subjected during 5 min to the cage, which was discarded from the statistical analysis due to habituation to the cage conditions. Twenty-five hours later, the rats were evaluated during 5 min (test session, day 28 of treatment), where the following variables were evaluated: (a) number of crossings, as per movement of at least three quarters of body from one square to another by the rat (b) number and time spent rearing, when the rat assumed a vertical posture relative to the floor, supported on its hind limbs; and (c) number and time spent self-grooming, an indicator of the motivational state of the animal [19]. After this test, the rats were evaluated on the rota-rod test.

2.7.2. Rota-rod Test

The rats were trained on a rota-rod (LE 8300, Leticia LSI, Panlab Scientific Instruments, Barcelona, Spain) for 5 consecutive days at a speed of 18 rotations per minute before the treatments were given. The latency to fall from the rota-rod was recorded for 5 minutes, which was the time that elapsed after the rat was placed on the rod until it fell. This test was conducted to identify changes in motor coordination and balance [20]. After the rota-rod session, the rats were evaluated in the swim test.

2.7.3. Swim Test

The rats were individually placed for 5 min in a rectangular pool (26 cm × 29 cm × 50 cm) that was filled with water (25°C ± 1°C). The depth of the water was adjusted so that the rat could touch the bottom of the pool with one or both of its hindlimbs and tail. None of the animals drowned. The variable that was evaluated in this test was spin behavior, which is considered an indicator of motor incoordination [15]. Spin behavior was defined as periods in which the rat swam on its own axis, without horizontal displacements [15,16].

All the locomotor activity and swim test sessions were videotaped. Two blind independent observers quantified the variables until they reached a consensus of at least

95%. Spin behavior in the swim test was evaluated in the videos and automatically analyzed using ANYmaze 4.73 software (Stoelting, Wood Dale, IL, USA).

2.8. Statistical Analysis

The data were analyzed using one-way ANOVA for independent groups. When significant differences of $p \leq 0.05$ were found in the ANOVA, Student-Newman-Keuls post hoc test was performed. All the data were presented as the mean ± standard error (SEM) of each variable. The statistical program used to carry out the test was SigmaStat 4.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Open-Field Test

3.1.1. Number of Crossings

The number of crossings revealed significant differences [$F(7,48) = 14.93$; $p = 0.001$] between groups. The post hoc test revealed that Cass2 and Cass3 groups significantly had a greater crossing in comparison to the Veh and the additional experimental groups. That effect was prevented by co-administration of resveratrol (Cass2+RV and Cass3+RV groups). No significant differences were detected between Cass1 and Veh group (e.g. Figure 1).

3.1.2. Number and time of Rearing

The number of rearing showed significant differences [$F(7,48) = 15.20$; $p = 0.001$] according to the treatments. The post hoc test revealed that this variable was significantly higher in Cass2 and Cass3 groups, in comparison to Veh, RV, and Cass1 groups. The effect produced by Cass 2 and Cass3 was prevented by RV. Similarly, the analysis of time spent in rearing revealed significant differences [$F(7,48) = 6.36$; $p = 0.001$] between treatments. The post hoc test revealed that time spent in rearing was higher in Cass2 and Cass3 groups, in comparison to Veh, RV, and Cass1 groups. That effect produced by Cass2 and Cass3 on time spent in rearing was prevented by RV, as shown in Table 1.

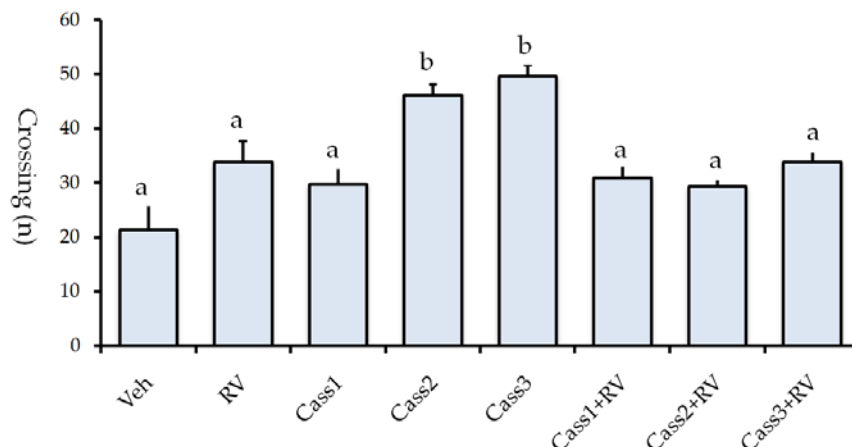


Figure 1. Number of crossings in the open field test. Veh, vehicle; RV, 10.7 mg/kg resveratrol; Cass1, 7.14g/kg cassava; Cass2, 14.28 mg/kg cassava; and Cass3, 28.56 g/kg cassava; n, number. Different letters mean statistical differences between groups ($P < 0.05$). One-way ANOVA, post hoc Student-Newman-Keuls

Table 1. Rearing evaluated in the open field test

Groups	Number of rearing	Time spent in rearing (s)
Veh	17.14 ± 1.51 ^a	30.29 ± 2.39 ^a
RV	18.85 ± 1.33 ^a	24.51 ± 3.84 ^a
Cass1	20.01 ± 1.02 ^a	30.27 ± 1.92 ^a
Cass2	27.42 ± 1.21 ^b	37.36 ± 2.67 ^b
Cass3	33.14 ± 2.17 ^b	44.58 ± 3.26 ^b
Cass1+RV	20.85 ± 1.31 ^a	29.84 ± 1.81 ^a
Cass2+RV	16.14 ± 1.11 ^a	31.13 ± 2.61 ^a
Cass3+RV	19.71 ± 1.79 ^a	32.41 ± 1.93 ^a

Veh, vehicle; RV, resveratrol; Cass1, 7.14g/kg cassava; Cass2, 14.28 mg/kg cassava; and Cass3, 28.56 g/kg cassava; s, seconds. * $p < 0.05$ vs Veh, and all experimental groups. Different letters mean statistical differences between groups ($P < 0.05$). One-way ANOVA, post hoc Student-Newman-Keuls.

Table 2. Grooming evaluated in the open field test

Groups	Number of grooming	Time spent in grooming (s)
Veh	9.28 ± 1.71	30.31 ± 2.78
RV	9.85 ± 1.16	34.41 ± 3.34
Cass1	9.01 ± 1.09	36.69 ± 3.72
Cass2	10.01 ± 0.92	36.04 ± 2.54
Cass3	11.57 ± 1.06	40.21 ± 4.83
Cass1+RV	8.09 ± 1.07	34.17 ± 2.73
Cass2+RV	9.28 ± 1.19	27.65 ± 4.66
Cass3+RV	9.43 ± 0.99	31.30 ± 2.97

Veh, vehicle; RV, resveratrol; Cass1, 7.14g/kg cassava; Cass2, 14.28 mg/kg cassava; and Cass3, 28.56 g/kg cassava; s, seconds. No significant differences in number and time spent in grooming were found between treatments. One-way ANOVA.

3.1.3. Number and Time Spent in Grooming

The number of grooming sessions did not show significant differences [$F(7,48) = 0.75$; $p = 0.631$] according to the treatments. Likewise, the analysis of time spent in grooming was not significantly [$F(7,48) = 6.36$; $p = 0.068$]

modified by the treatments, as shown in Table 2.

3.2. Rota-rod

3.2.1. Latency to Fall

The analysis of this variable revealed significant differences [$F(7,48) = 46.66$; $p = 0.001$] between groups. The post hoc test revealed that Cass2 and Cass3 groups significantly had a lower latency to fall from rota-rod, in comparison to the Veh and the additional experimental groups. That effect could be attributed to co-administration of resveratrol (Cass2+RV and Cass3+RV groups). No significant differences were detected in Cass1 in comparison to the Veh group, nor between Cass2 and Cass3 groups on this variable (e.g. Figure 2).

3.3. Swim Test

3.3.1. Spin Behavior

The low number of rats that displayed the spin behavior in the experimental groups did not permit realize a statistical analysis. The Veh and RV groups did not display spin behavior in the swim test as it was expected considering that both groups were free of any toxic substance. However, the rats treated with different doses of cassava juice displayed this behavior. The higher number of rats that show spinning behavior were those from the Cass2 and Cass3 groups. Interestingly, in groups treated with Cass2 and Cass3 plus resveratrol, a lower number of animals showed spin behavior: Cass1, 42.8%; Cass2, 85.7%; Cass3, 71.42; Cass1+RV, 42.8%; Cass2+RV, 28.6%; and Cass3+RV, 42.8%. (e.g. Figure 3).

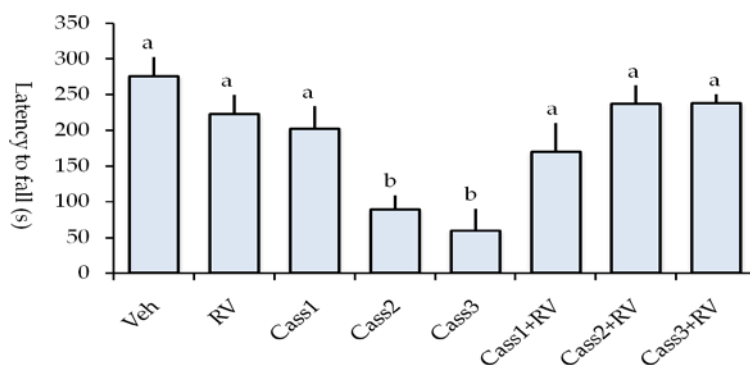


Figure 2. Latency to fall in the rota-rod test. Veh, vehicle; RV, 10.7 mg/kg resveratrol; Cass1, 7.14g/kg cassava; Cass2, 14.28 mg/kg cassava; and Cass3, 28.56 g/kg cassava; s, seconds. Different letters mean statistical differences between groups ($P < 0.05$). One-way ANOVA, post hoc Student-Newman-Keuls

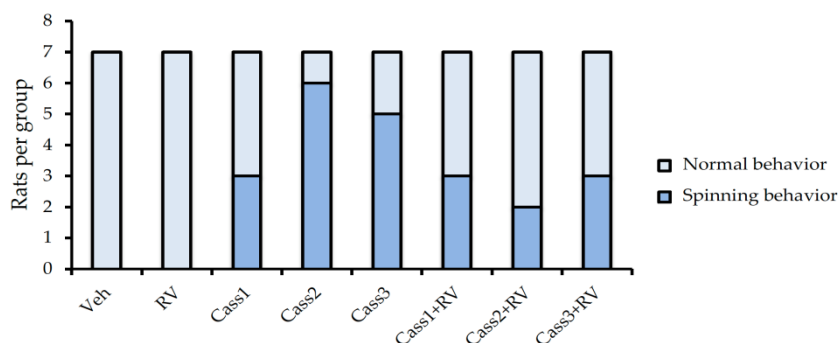


Figure 3. Number of rats that displayed spinning behavior in the swim test. Veh, vehicle; RV, 10.7 mg/kg resveratrol; Cass1, 7.14g/kg cassava; Cass2, 14.28 mg/kg cassava; and Cass3, 28.56 g/kg cassava

4. Discussion

The aim of the present study was to evaluate whether resveratrol prevents motor impairment associated to cassava juice consumption [5,6]. The results indicate that the administration of resveratrol exerts a protective effect against the motor impairment induced by the chronic consumption of cassava juice, which could be related to the antioxidant and anti-inflammatory effects in the central nervous system attributed to resveratrol.

The open-field test was initially used to assess the degree of "emotionality" in rats [21]. However, this test can also be used to assess the effects of various substances on anxiety levels, as well as the non-specific effects of drugs and diverse molecules on locomotor activity [22]. Additionally, it is used to measure motor skills that depend on the maturation and adequate functioning of the central nervous system, as well as the integrity of the motor pathways that control movement [23]. Some of the parameters that this test allows to evaluate are motor hypoactivity or hyperactivity (a decrease or increase in the number of crossing), changes in exploration (time spent rearing), and motivation (time spent grooming) [24,25]. In the present study, the activity in the open-field test was evaluated to identify motor effects associated with the treatments, which involves memory consolidation processes acquired in the habituation session [26]. In this case, the Cass2 and Cass3 groups showed a higher number of crossings as compared with vehicle group, which suggest a potential memory impairment in which the rat does not recognize the conditions of the cage, increasing the motor activity respect to vehicle-treated group as consequence of exploration in unrecognized environment. The negative effects reported in rats treated with the same doses of cassava (for example, increased locomotion and rearing) were not evident in the rats that received the co-administration of cassava and resveratrol; this suggests that rats maintain their normal exploratory capacity and adequate memory consolidation when identifying test conditions in the open-field test [27]. Considering that *in vitro* and *in vivo* studies had confirmed that resveratrol crosses the blood-brain-barrier [28] it is possible suggest that, in present study, the reduction in crossings could be associated with the antioxidant actions of resveratrol on hippocampal neurons. It probably prevented neuronal damage induced by the components of cassava juice, allowing memory consolidation by maintaining the proper functioning of specific neurotransmitter systems such as the dopaminergic system. Additionally, resveratrol can increase the expression of FOXO3, which is a first-line defense gene against oxidative stress in dopaminergic cell [29]. Therefore, it is suggested that resveratrol could facilitate consolidation of memory; and hence thus, the rats remember the characteristics of the locomotor activity cage, and this reduces exploration. Contrarily, when rats have a deterioration in memory (i.e., in Cass2 and Cass3 groups), they explore the cage as a new and unfamiliar environment.

Grooming is an indicator of the motivational and emotional state of the rat. Time spent grooming increases in rats subjected to mild stress, while it decreases under conditions of severe stress as compared to control animals [30-33]. In the present study, all the rats maintained

grooming behavior independently of the treatment. This suggests that cassava molecules and resveratrol could not target specific neurotransmitter pathways involved in the control of this behavior.

On the other hand, rearing is considered an indicator of exploration that implies a high degree of motor coordination. This behavior is increased in rats with neuronal damage in the dorsal hippocampus and is evidently associated with a state of hyperactivity and motor incoordination [34,35,36,37]. In turn, this represents a response to novel stimuli, reflecting independent biological functions in which memory participates; this involves cognitive processes and adaptive changes in both short- and long-term memory [23]. In this study, a greater rearing behavior (number and time) was observed in the rats of the group Cass3; suggesting that the rats did not habituate to the cage conditions during the pretest session, that is, they showed high exploration during the test because of a memory impairment. This effect in accordance with that reported in previous studies in which rats treated with the same doses of cassava juice showed an increase in this variable, associated with memory loss [35,38,39,40,41]. Interestingly, the group treated simultaneously with Cass3 plus resveratrol prevented the high rearing in this behavioral test. These data suggest that resveratrol exerted protection against negative effects produced by cassava juice, which could have been associated with a reduced apoptosis and increased expression of the brain-derived neurotrophic factor in specific brain structures as hippocampus [6,41].

One of the important equipment used in the studies on animal behavior is the rota-rod test, originally described by Dunham and Miya in 1957 [42]. Animals with normal motor coordination remain longer time on the roller in motion than those that have decreased activity and motor incoordination [43]. However, its use requires a habituation that allows the strengthening of the animal's memory of walking, coordination, and resistance [44,45].

In our study, the rats from Cass2 and Cass3 groups showed a shorter latency to fall in the rota-rod, which indicate a clear deterioration of motor coordination. Interestingly, the effects produced by cassava was significantly prevented by co-administration of resveratrol (Cass2+RV and Cass4+RV groups), reaching up similar values in the vehicle group. This shows that the animals in these groups presented normal motor coordination, which allowed them to remain on the moving roller for longer time. Similar effects have been reported by Mancuso et al. (2014) [46] in SOD1(G93A) ALS mice, which develop paralysis in one or more limbs within a few weeks of age, consequently developing motor incoordination. In that study, the treatment with resveratrol, starting at 12 weeks of age, prevented the motor deterioration and increased the motor coordination in the rota-rod, which was associated with improvement in the function and survival of spinal motor neuron. Therefore, resveratrol may be a promising therapy for the treatment of motor impairment.

The swimming test has been used to investigate toxic effects on the central nervous system, in addition to drugs that damage the vestibular system; and neurotoxic drugs that produce motor incoordination in animals [47,48]. Swimming consists of certain fundamental aspects: balance that keeps the animal constantly on one axis, motor activity that allows the animal to move and float,

coordination between muscles that generates stability in the water, and a memory that eventually allows this behavior to be performed again [48]. In our research group, this technique was used in rats for the evaluation of motor impairment associated with the consumption of potentially toxic substances such as cassava, considering spin behavior as an indicator of motor toxicity [15,19]. Spin behavior indicates incoordination and imbalance that is attributed to the damage caused by substances with neurotoxic capacity [16]. This behavior has also manifested in rats fed *Dioon spinulosum* seeds and was associated with a decrease in the number of neurons in the CA1 area of the hippocampus [16,37]. This test is a type of methodology with greater sensitivity since the motor functions involved in swimming require a set of specific muscles, reflexes, and brain regions for the entire procedure [49]. In previous studies, it was reported that the administration of cassava juice promotes lateral swimming and spin behaviors, which are proposed as an indicator of motor incoordination [50]. In another study, it was shown that the rats treated with cassava juice develop spin behavior and lateral swimming, which was associated with one of the cassava metabolites linamarin. When this molecule is metabolized, it produces cyanide, which causes a series of changes in the biochemistry of the brain and oxidative stress that predisposes to neuronal death and, subsequently, to alterations in motor function [51]. In this sense, exposure to cyanide in rats produces neuro-degeneration in the spinal cord and ganglia in the central nervous system, in addition to inhibiting the electron transport chain, thus causing the generation of reactive oxygen species, which cause oxidative stress and neuroinflammation, mechanisms involved in neurodegeneration and motor and cognitive disability [39,52]. In present study, statistical analysis of spin behavior was not performed considering the low number of animals and the low number of animals that showed this behavior. However, the higher number of rats that presented this behavior were those included in Cass2 and Cass3 groups, while a reduced number of rats that showed spinning behavior were from those groups co-administered with resveratrol (Cass2 and Cass3 plus RV), showing the protective effect of resveratrol against motor alterations induced by cassava juice. The motor incoordination identified in the swim test in rats treated with cassava juice has been reported in previous studies, where the treatment with *Ginkgo biloba* extracts prevented its negative effects on motor incoordination, due to high content of flavonoids [40]. On the other hand, resveratrol, like *Ginkgo biloba* extract, has a high antioxidant activity due to the presence of flavonoids that act beneficially on neurodegenerative diseases [53]. In addition, the antioxidant capacity of resveratrol has been evidenced in vitro, genetic, and enzymatic studies, where it was observed that deacetylase Sir2/SIRT1, an enzyme that promotes resistance to stress and aging, is the target of resveratrol. This increases the activity of the endogenous antioxidant pathway and activates the production of sirtuins, with an improvement in physical capacity of the animals [54].

In present study, it was shown that the administration of resveratrol prevented the manifestation of spin behavior in the swim test. This behavior suggests the possibility of motor damage caused by cassava juice, mainly due to its metabolite linamarin [55]. Although the mechanism of

action underlying the protective effects of resveratrol on behavioral variables was not explored in the present study, it is suggested that once resveratrol is administered orally in rats, it is rapidly absorbed and metabolized by glucuronides or sulfate conjugates mainly by phase II enzymes and is distributed to various organs [56,57]. At the intestinal level, resveratrol is absorbed in its glycosylated form, either by passive diffusion or by forming complexes with membrane transporters, reaching the blood 30 minutes after oral intake [58]. Once in the blood, resveratrol can be transported bound to albumin and lipoproteins such as LDL (low-density lipoproteins), and the complex formed can be dissociated in cell membranes that contain receptors for albumin and LDL; in the case of the brain, megalin leaves the resveratrol free and allows it to enter cells [59]. Taking into consideration the type of damage caused by cassava juice and studies that demonstrate the protective effect of resveratrol, our study proposes that there may be a mechanism of action involving the activation of AMPK when resveratrol is co-administered with cassava juice. AMPK is a protein that is involved in the energy balance not only of specific cells but also of the entire organism in response to the availability of some nutrients or situations of metabolic stress that act on the central nervous system. It is activated by an increase in the AMP/ATP ratio (oxidative stress) [60]. Resveratrol has been shown to activate AMPK signaling and the neuronal metabolism, playing an important role in the neuronal energy metabolism at two levels: a) AMPK activation in hypothalamic neurons plays a central regulatory role in energy expenditure; and b) at the cellular level, AMPK functions as an important energy sensor where it detects intracellular calcium mobilization [61,62]. Sirtuins or class III histone deacetylases are NAD⁺-dependent proteins that act on processes in the mitochondria, regulating energy metabolism. In one study, it was shown that SIRT1 is expressed in hippocampal neurons and that resveratrol is an activator of this protein [62,63] when there is oxidative stress; by increasing SIRT1 expression, resveratrol regulates the normal cognitive functions of mice. It has been shown that SIRT1 is essential for the maintenance of synaptic plasticity [63]. All these effects at the cellular and molecular level could contribute to the neuronal mechanisms underlying the neuroprotective effects of resveratrol, which should be considered in future studies.

5. Conclusions

The co-administration of resveratrol exerts a protective effect against motor impairment induced by cassava juice in adult rats, which could be associated with the antioxidant effects attributed to resveratrol. Thus, the consumption of resveratrol may contribute to prevent neurological disorders as Tropical Ataxic Neuropathy and Konzo disease associated with chronic cassava intake and, possibly, of other toxic substances.

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