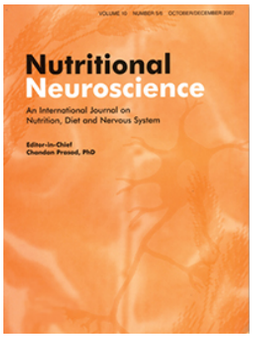

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Mauritia flexuosa L. protects against deficits in memory acquisition and oxidative stress in rat hippocampus induced by methylmercury exposure

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Objective: Methylmercury (MeHg) is the most toxic form of mercury that can affect humans through the food chain by bioaccumulation. Human organism is capable of triggering visual and cognitive disorders, neurodegeneration, as well as increased production of reactive species of O2 and depletion of natural anti-oxidant agents. In this context, *Mauritia flexuosa* L., a fruit rich in compounds with anti-oxidant properties, emerged as an important strategy to prevent the MeHg damages. So, this work has aimed to elucidate the protective effect of Mauritia flexuosa L. on the damage caused by the exposure of rats to MeHg.
Methods: In order to evaluate the effect of MeHg on rat aversive memory acquisition and panic-like behavior, we have used elevated T-maze apparatus and after behavioral test, the hippocampus was removed to perfom lipid peroxidation.
Results: Our results demonstrated that the exposure to MeHg caused deficits in inhibitory avoidance acquisition (aversive conditioning) and in the learning process, and increased levels of lipid peroxidation in hippocampus tissue. However, the pretreatment with feed enriched with *Mauritia flexuosa* L. showed a protective effect against cognitive deficits caused by MeHg and also prevented the occurrence of cytoplasmic membrane damage induced by lipid peroxidation in the hippocampal region.
Discussion: Therefore, this study suggests that *Mauritia flexuosa* L. represents an important strategy to prevent neurocytotoxics and behavioral effects of MeHg.

Keywords: *Mauritia flexuosa* L., Methylmercury, Hippocampus, Oxidative stress, Memory acquisition

Introduction

The Amazon region represents an important source of natural products with recognized therapeutic potential. A crescent number of studies have demonstrated that plants and fruit from Amazon forest showed pharmacological activity in different experimental models.[1–4] Although with recognized potential, few studies describe the possible utilization of Amazon plants for treatment or prevention of brain injuries induced by neurotoxic environmental contaminants such as methylmercury (MeHg).[5–10] In fact, most pharmacological studies about Amazon plants are mainly focused on its characterization as anti-inflammatory or anti-oxidant agents.[9–15]

In the Amazon region, several fruits are commonly consumed by local communities and among these include the fruit from *Mauritia flexuosa* L. (MF)

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popularly known as Buriti. MF belongs to the *Araceae* family, found in North and South America, specifically in the Amazon region, which grows naturally in flooded soils.[16–21] MF fruit is an ellipsoid drupe oval covered with scales dark reddish measuring between 5 and 7 cm in diameter. Populations living at Amazon region commonly use the pulp of MF fruit as part of the diet or for treatment of different diseases.[14,15,17] Studies have demonstrated that MF fruit represents a natural source of anti-oxidants such as vitamin A, carotenoids, and tocopherol.[14,17,22] Although few studies have described the pharmacological properties of MF fruit, some works have demonstrated its possible action in diseases such as xerophtalmia or in injuries associated with oxidative stress.[23]

Formation of reactive oxygen species (ROS) represents an important mechanism associated with the central nervous system diseases including neurobehavioral alterations such as anxiety, ataxia, and memory impairments.[24–28] It is well documented that events related to heavy metal intoxication can also induce oxidative stress in different brain areas.[29–33]

Several reports have demonstrated that environmental contamination with MeHg represents risk for populations living at regions of gold-digging.[20,21,34–40] Our group has previously demonstrated that Amazon riverside populations showed increased MeHg levels in their hair. These data strongly suggest human intoxication with the metal.[39,41] In addition, we have demonstrated in this population a positive association between elevated MeHg levels and decreased activity of anti-oxidant enzymes.[39,41] Studies utilizing animal models showed an association between neurobehavioral disturbances and oxidative stress induced by MeHg intoxication.[42–48] In fact, previous works suggested that memory impairment, anxiety-like behavior, visual and motor dysfunctions are important signals of MeHg toxicity.[24–28] It is well described that some brain areas controlling learn and memory acquisition represent important targets of MeHg toxicity.[27] Hippocampus is a limbic brain structure close associated with control of animal memory.[27,49] Previous report describes that rats prenatally exposed to MeHg presents significant behavior impairment associated with increased oxidative stress in the hippocampus.[27, 50–52] Thus, considering the known mechanism associated with MeHg toxicity in the central nervous system and phytochemical description of MF, in the present study we evaluated whether a dietary enriched with MF fruit is able to prevent the behavioral and biochemical alterations induced by MeHg exposure.

Methods

Plant material

Mauritia flexuosa fruit was collected from local farm localized at Castanhal City, Pará State, Brazil. The plant and fruit was identified in the University Federal of Pará. MF-enriched food was produced by mixing regular commercial chow (23% gross protein, 4% ethereal extract, 5% raw fibrous, 10% mineral matter, 1.3% calcium, and 0.85% phosphorus) and fruit pulp (1:1 g/g) with ultrapure water. The mixture was compressed into pellets and dried by warming at 40°C for 2 h. Finally, the enriched ration was given to the animals after cooling to room temperature.

Animals

All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* from the Ethic Commit of the Federal University of Pará (UFPa) protocol number 122–13. Male Wistar rats weighing 250–280 g (3 months old) were housed at constant room temperature (20–22°C) with light cycle of 12 h/day and free access to food and water.

Experimental groups treatments

Animals ($n = 28$) were divided in two different diet groups, one exposed to food constituted with commercial ration ($n = 14$) and other with supplemented ration ($n = 14$) for 7 days before the MeHg exposure. Each animal group had free access to commercial or enriched chow during experimental period. The analysis of body mass was carried out by five consecutive days. After diet period, two sub-groups, commercial ration group ($n = 7$) and supplemented ration group ($n = 7$), were exposed to 5 mg/kg/day methylmercury chloride (MeHg) by gavage during three consecutive days. The animals not exposed to MeHg received only saline solution by oral administration. This step was followed by 5 days of acclimatization before behavioral or biochemical analysis.

Elevated 'T'-maze (ETM)

In order to evaluate the effect of MeHg exposure on rat aversive memory acquisition and panic-like behavior, we have used elevated T-maze apparatus (ETM) as described previously.[53–55] The ETM is an adaptation of the plus-maze apparatus where closed arms were substituted by a shielding contraption.[53] The inhibitory avoidance test was started with the placement of the animal into the distal portion of the closed arm.

The animal head was turned in the open arm direction and latency of animal exit from closed arm (registered when the animal had stepped its four legs outside of the closed arm) was recorded. After latency evaluation, the animal was taken out of the maze for 30 seconds for restart the next this two avoidance tests. These inhibitory avoidance trials were named baseline avoidance, avoidance 1 and avoidance 2 tests, respectively. After the last avoidance trial, the animal was taken out of the maze for 30 seconds and it was placed in the end of open arm for the escape trial. The escape latency from open arm (registered when the animal stepped with its four legs in the closed arm) was recorded.[53,55]

Elevated plus-maze (EPM)

Thirty minutes after ETM test, the animals were submitted to plus-maze test. This behavior evaluation was performed in order to verify the effect of MeHg on the anxiety-like behavior. The plus-maze used in this study was constructed of wood with two open arms (30 × 10 cm) with 1 cm border protection, and two closed arms with 15 cm borders arranged perpendicular to the open arms. The whole apparatus was elevated 50 cm from the floor. Animals were placed individually in the center of the maze with the head turned to one of the closed arms and their behavior was freely recorded for 5 minutes in the EPM. Entries in the closed or open arms were recorded only when the animal was positioned with all four paws in one arm. 'Ethological' measures included the frequencies of head-dipping, stretch-attend postures, rearing, and grooming. The session was recorded by a 30 fps digital camera interfaced via USB on a digital computer with the aid of the program Debut Video Capture Software version 1.49. Videos were later analyzed using the software X-Plo-Rat 2005 (http://scotty.ffclrp.usp.br).

Lipid peroxidation test

After behavioral test, the animals were deeply anesthetized and hippocampus was quickly removed. The tissue was homogenized in phosphate buffer saline (pH 7.4) at 4°C. The homogenate was centrifuged at 3000 rpm for 5 minutes and the supernatant was used for the biochemical evaluation. The analysis of the lipid peroxidation was carried out based on standard curve concentrations of malondialdehyde, measured by the absorbance at a wavelength of 535 nm in accordance to previous studies.[56,57]

Statistical analyses

Behavioral data were expressed as media ± standard error and the biochemical data expressed as media ± standard derivation. Normal distribution of the data was confirmed by the Shapiro–Wilk test. Media of values were compared using one-way ANOVA followed by Bonferroni post-test. Statistical analysis was carried out using BioEstat Software 5.0 software and P-values <0.05 were considered significant.

Results

Memory acquisition test

Before all behavioral evaluations, body weight of control and diet groups was measured and our results demonstrated that neither MeHg exposure nor MF-enriched diet has induced significant changes in the body weight of animals (Table 1).

Memory acquisition was evaluated utilizing the elevated T-maze test as described in the method. Control group showed increased latency period during avoidance 2 test when compared with avoidance 1. This result suggests memory acquisition in the control group (Fig. 1). On the other hand, animals treated with MeHg showed low latency in the closed arm when submitted to avoidance 2 tests. These data indicating lack of memory acquisition induced by MeHg exposure. As observed in Fig. 1, animals intoxicated with MeHg and feed with MF-enriched ration did not show lack of memory acquisition. Although we observed that MF-enriched diet has evoked memory acquisition already from avoidance 1 test, no difference was observed in avoidance 2 test when compared with control (Fig. 1).

Anxiety and motor activity evaluation by EPM task

It is well documented that anxiety-like behavior or fear/panic-like behavior can influence memory acquisition in rodents. In this way, we tried to assess whether memory impairment induced by MeHg could be associated with anxiogenic-like behavior. Our results demonstrated that both MF-enriched diet and MeHg

Table 1 Analysis of body mass gain. Data were shown as mean ± standard error and analyzed by one-way ANOVA followed by Bonferroni post-test

	Before the treatment with ration	Gavage Day 1	Gavage Day 2	Gavage Day 3	Before behavior test
Normal ration + saline (0.9%)	243.66 ± 4.37	244.33 ± 4	245.16 ± 4.14	246.16 ± 4	246.66 ± 4.1
MF-enriched ration + saline (0.9%)	213.25 ± 6.18	223.25 ± 7	218.75 ± 8.22	224.50 ± 7.84	217.25 ± 6.34
Normal ration + MeHg 5 mg/kg	217.0 ± 4.50	221.4 ± 5.26	221.2 ± 5.16	220.0 ± 5.05	220.0 ± 5.34
MF-enriched ration + MeHg 5 mg/kg	211.4 ± 5.88	217.6 ± 5.87	214.2 ± 5.01	208.8 ± 6.79	202.4 ± 7.44

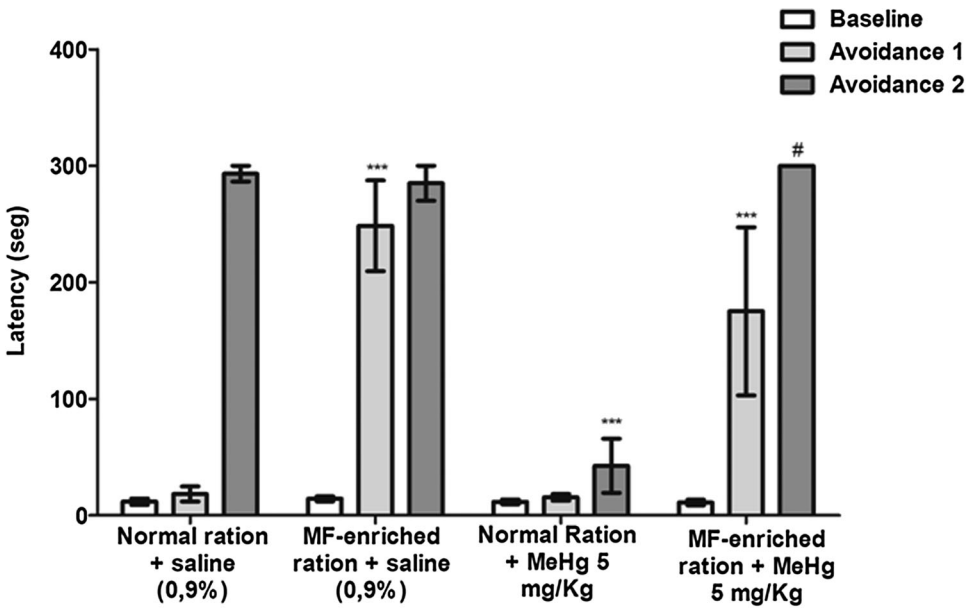

Figure 1 Analysis of inhibitory avoidance acquistion was performed from three replicates (baseline, avoidance 1, avoidance 2) at intervals of 30 seconds. It was recorded the latency in LTE. Data were shown as mean ± standard error and analyzed by one-way ANOVA followed by Bonferroni post-test, with $P < 0.001$ as significant. ***Compared to normal diet group + saline (0.9%); compared to control diet group + MeHg (5 mg/kg).

did not evoke significant alterations in anxiogenic-like parameters such as grooming, head-dipping, and SAP or fear/panic-like parameter (latency in the open arm) (Fig. 2). Data from horizontal displacement evaluation suggested no motor alterations in rats exposed to MeHg (Fig. 3).

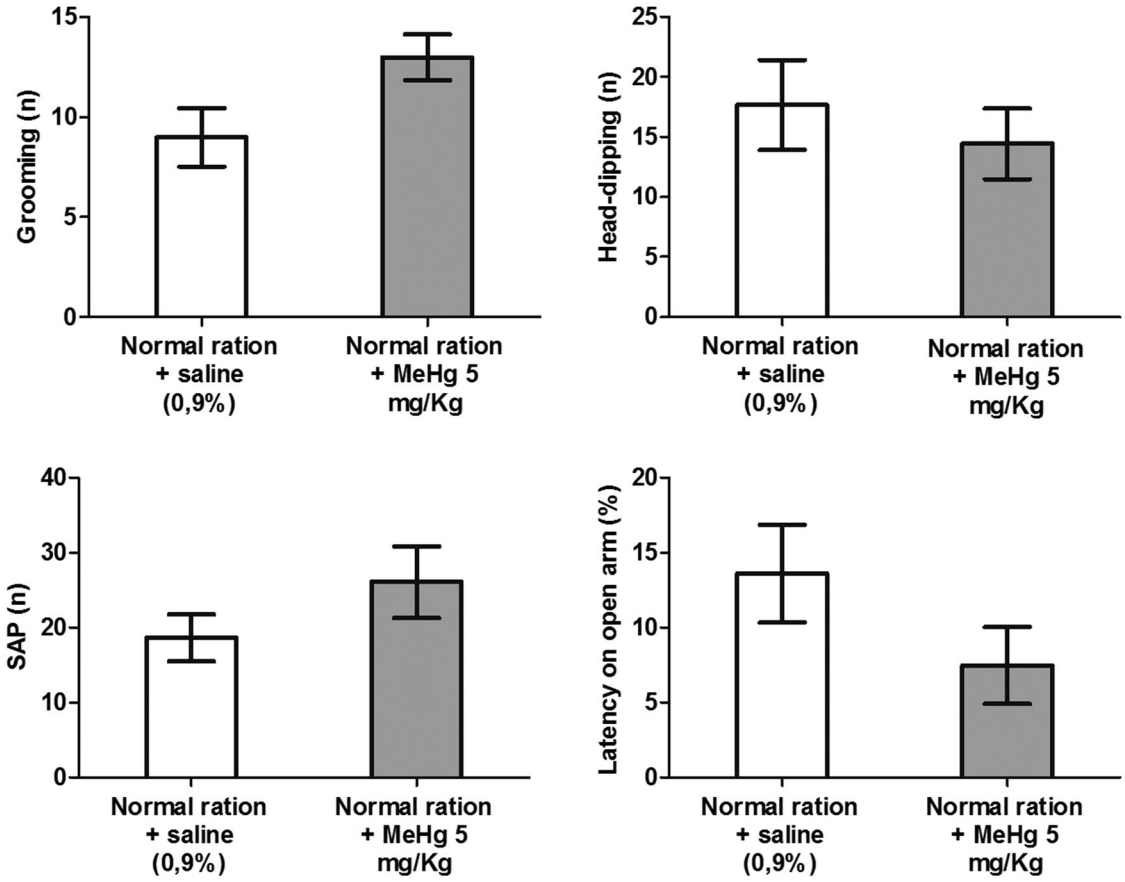

Figure 2 Analysis of the number of grooming, head-dipping, stretch-attend postures (SAP) and time spent on the open arm in the EPM. Data were shown as mean ± standard error and analyzed one-way ANOVA followed by Bonferroni post-test.

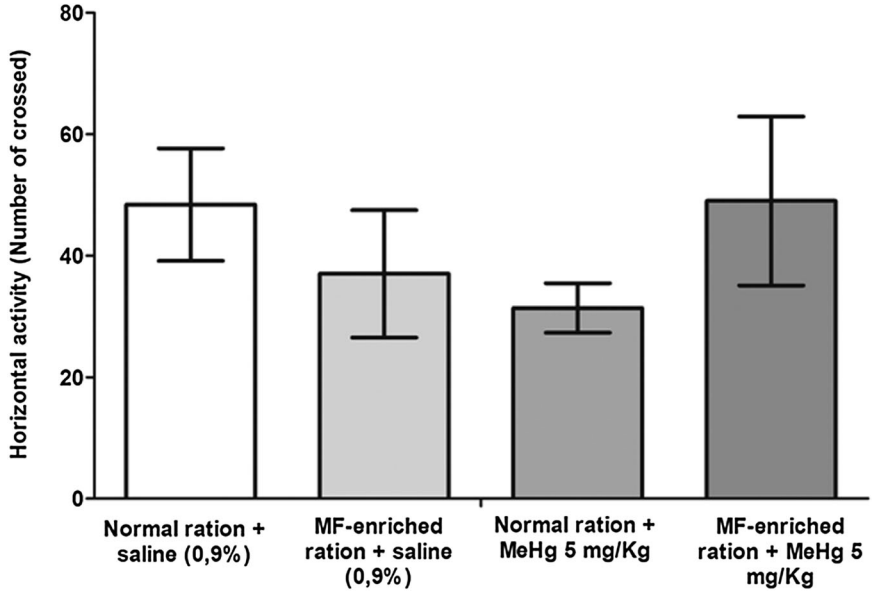

Figure 3 Locomotor activity (number of crossed) in the EPM. Data were shown as mean ± standard error and analyzed by one-way ANOVA followed by Bonferroni post-test.

MeHg induces lipid peroxidation in rat hippocampus

The results presented above suggested that MeHg affected specifically aversive memory acquisition without induce changes in motor, anxiety, or panic-like behavior. It is well documented that hippocampus represents an important cerebral structure that controls memory acquisition. In this way, we have verified whether the deficit on the memory acquisition induced by MeHg was associated with oxidative stress.

Our results showed that hippocampus of animals intoxicated with MeHg showed about 150% of TBARs production when compared with control group. On the other hand, rats intoxicated with MeHg and feed with MF-enriched ration did not show increased hippocampal TBARs levels when compared with control (Fig. 4).

Discussion

Riverside Amazon population utilizes regional fruit as component of supplemental dietary[16,17,20–22] and previous studies describe that fruits form Amazon florets are used by local community for treatment of different diseases.[15,17,58–60] In the present work, we demonstrated, for the first time, that MF fruit dietary prevents memory impairment and oxidative stress in hippocampal tissue induced by MeHg in rats.

Several reports have described that MeHg represents an environmental biohazard for Amazon riverside populations living at gold-digging areas.[20,21,35–40] It

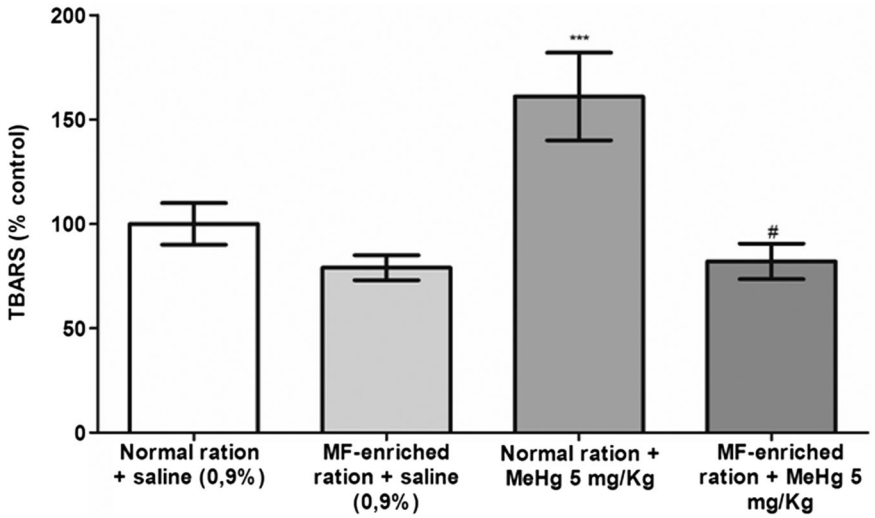

Figure 4 Hippocampus lipid peroxidation. Data were shown as mean ± standard error and analyzed by one-way ANOVA followed by Tukey post-test with $P < 0.05$ as significant. ***Compared with the normal ration + saline (0.9%); #compared with the normal ration + MeHg (5 mg/kg/day).

is also well described that the central nervous system is a potential target of MeHg intoxication.[42–46,48,61,62] Although studies demonstrated that behavioral alterations induced by MeHg can be associated with toxicological actions in humans and animal models,[8,31,33,46,63–66] few works have evaluated the potential effect of Amazon fruits against behavioral toxicity induced by MeHg.

In the present study, we demonstrated that MeHg induces severe deficits in memory acquisition in rats (Fig. 1). These results are in agreement with previous reports showing pronounced memory impairment in rodents exposed to acute doses of MeHg.[26,67] Studies utilizing animal models describe MeHg affecting different brain functions, including anxiety-like behavior, motor activity, and panic-related behavior.[8,46,67,68] Our results have demonstrated no significant alterations in anxiety, motor, and panic indicating parameters induced by MeHg. Taken together these results suggest that MeHg had induced a highlighted memory acquisition impairment more than other behavioral changes. Our data showed that *M. flexuosa* fruit dietary exerted a protector action against memory lack induced by MeHg exposure (Fig. 1). We administered orally the fruit in combination with commercial ration in order to simulate the natural consumption of *M. flexuosa* fruit by Amazon population. Our results also suggest that *M. flexuosa* fruit intake can exerts its protector effect by blocking the oxidative stress induced by MeHg in the brain hippocampus (Figure 4). In fact, ROS generation with consequent lipid peroxidation represents an important mechanism associated with several brain disorders, including toxicological action of heavy metals as MeHg.[30,31,69–72] Studies demonstrated that memory impairment also can be attributed to oxidative stress in hippocampal tissue.[73,74] In fact, precise mechanism involved in the memory impairment induced by oxidative stress in the brain are not fully understood; but recent works have pointed that oxidative stress inhibits neuron formation as well as is able to alter the maintenance of dendritic network in the hippocampus.[75,76] New neurons formation and hippocampal dendritic network integrity are crucial to provide the synaptic plasticity needed for learning and formation of memories. In the present study, we have demonstrated that MeHg exposure evokes oxidative stress in rat hippocampus as well as we have showed that *M. flexuosa* fruit diet prevents against this effect (Figure 8). These results are the first to demonstrate that oral administration of Amazon fruit could evoke protective effect against MeHg toxicity.

It is well described that several Amazon fruit presents in its chemical constitution several anti-oxidant agents; and it is also known that *M. flexuosa* has a high concentration of beta-carotene in its fruit (about 70%).[14,22,23,49,77] A recent work utilizing domestic processing of carotenoids rich vegetables has demonstrated that wet heat processing at 50°C for 3 hours do not reduces concentration and anti-oxidant activity of carotenoids in the analyzed matrix.[78] In the present study, *M. flexuosa* pulp fruit was heated at 40°C for 2 hours during enriched chow preparation. Thus, although we do not rejected the hypothesis that other anti-oxidants present in the fruit pulp may have suffered changes during its processing, the procedures used in the present study seem not to be able to evoke significant changes on the carotenoids content present in the *M. flexuosa* enriched chow. In regard to nutritional parameters of enriched dietary, future bromatological analysis must be performed to characterize the caloric values associated with the *M. flexuosa* fruit enriched diet. At the moment, our data represent only the first pre-clinical validation of the *M. flexuosa* fruit use as protective against toxicity MeHg-induced on the CNS.

In conclusion, we demonstrated that *M. flexuosa* fruit has an efficient effect against behavioral and biochemical toxicity induced by MeHg; and *M. flexuosa* fruit could be alternative treatment to minimize the toxicological effects of MeHg in people living at regions of gold-digging.

Conclusion

This study showed, for the first time, the protect effect of *Mauritia flexuosa* L. (Buriti), considering the exposition of Wistar rats to 5 mg/kg of methylmercury induces deficits on inhibitory avoidance acquisition and aversive memory, hindering the learning process and increased the levels of lipidic peroxidation on hippocampus. However, pre-treatment with buriti-enriched ration was able to prevent damage both the behavioral and biochemical. Therefore, *Mauritia flexuosa* L. (Buriti) can be considered an important prevention strategy against high rates of mercury intoxication.

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Disclaimer statements

Contributors All authors contributed equally.

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Conflicts of interest There are no conflicts-of-interest.

Ethics approval The research has the approval of local Research Ethics Committee.

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