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Syzygium malaccense fruit supplementation protects mice brain against high-fat diet impairment and improves cognitive functions

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ABSTRACT

In this study, we supplemented a high-fat diet (HF) with 5% powder of freeze-dried *Syzygium malaccense* fruit (HS) and investigated proofs of peripheral insulin resistance, hippocampal AKT–GSK3-β–tau activation, learning/memory performance, and brain frontal lobe oxidative stress. The intake of HS did not prevent weight gain but promoted peripheral insulin sensitivity, improved AKT signaling in the hippocampus, which prevented the activation of GSK3-β and lowered tau phosphorylation induced in HF. The results from the Morris water maze cognitive test were consistent with the lower tau-phosphorylation, once the HS group showed lower latency in the acquisition phase and more times crossing the target quadrant when compared with HF. The HS diet improved brain antioxidant enzyme activities. Overall, the intervention with *S. malaccense* fruit to mice was effective in minimizing cognitive deficit caused by HF consumption and in preventing risk markers associated with Alzheimer's disease without reducing weight gain in mice.

1. Introduction

Accumulating evidence suggests that obesity might be associated with a higher risk of dementia and Alzheimer's disease (AD) occurrence in middle or late life (Profenno & Faraone, 2008; Profenno, Porsteinsson, & Faraone, 2010); and indicate insulin resistance as one among causative links between them (Profenno & Faraone, 2008). Furthermore, mechanistic studies report that diet-induced obesity enhances AD-associated pathological processes such as increased tau (microtubule-associated tau protein) phosphorylation in hippocampus and lowered cognitive functions due to insulin resistance (Jeon et al., 2012; Koga, Kojima, Kuwabara, & Yoshiyama, 2014; Ledreux, Wang, Schultzberg, Granholm, & Freeman, 2016; Schubert et al., 2003).

Impaired AKT signaling in neurons involves phosphorylation of tau, leading to the formation of neurofibrillary tangles (NFT), a marker of neuronal alterations in AD (Schubert et al., 2003). Physiologically, when insulin binds to its receptor in neurons, the signaling cascade phosphorylates AKT and GSK3-β in Serine 9, blocks tau phosphorylation, and then, prevents both the formation of NFT and further propagation to neuron losses (Cross, Alessi, Cohen, Andjelkovich, & Hemmings, 1995; Jolivalt et al., 2008). A study showed a 56% increased risk of AD in patients with a history of diabetes compared with nondiabetic subjects (Gudala, Bansal, Schifano, & Bhansali, 2013).

In the present study, we focus on how diets can play a role in preventing tau phosphorylation through obesity-related metabolic changes. For instance, the supplementation of diets with berries has been related to a better performance of cognitive-behavioral tasks and increased hippocampal plasticity in young and aged rodent feeding models (Casadesus et al., 2004; Rendeiro et al., 2012; Shukitt-Hale et al., 2015). Berry fruits contain flavonoids involved in several mechanisms related to prevention of insulin resistance, obesity and cognitive deficit as proven by previous animal studies (Batista et al., 2017; Rendeiro et al., 2012; Shukitt-Hale et al., 2015; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003). *Syzygium malaccense* (*Myrtaceae* family) fruit,

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popularly known as red-jambo, contains flavonoids such as cyanidin 3-glucoside (Batista, da Silva et al., 2017), an anthocyanin compound related with antioxidant activity, anti-obesogenic and anti-diabetogenic effects (Tsuda et al., 2003). Even though the fruit is underutilized and, so far, there is no information on the benefits of its consumption. Therefore, in the present study, we evaluated whether *Syzygium malaccense* fruit (SMF) supplementation has a role in preventing risk factors of cognitive deficits associated with high-fat diet intake. For this, peripheral and hippocampal insulin resistance associated with phosphorylation of tau, central oxidative stress, learning, and memory were investigated in adult mice.

2. Material and methods

2.1. Fruits' characteristics

In this experiment, we used the freeze-dried pulp and peel from SMF of the same stored batch of a previous study and whose chemical profile had been already assessed and published (Batista, da Silva et al., 2017). The main compounds in freeze-dried peel + pulp found in the previous study were: 33.7% total dietary fibers (27.9% insoluble and 5.6% soluble dietary fibers), cyanidin 3-glucoside, cyanidin 3,5-diglucoside, isorhamnetin 3-glucoside, kaempferol 3-glucoside, and procyanidins, among the 16 identified (poly)phenols (Batista, da Silva et al., 2017).

2.1.1. Polyphenols, antioxidant capacity, and anthocyanins analyses

To analyze phenolic compounds in the pulp + peel of red-jambo fruit, the freeze-dried powder was extracted in methanol: water: acetic acid (MeOH:H_2O:AcAc 85:15:0.5, v/v). Sample aliquots (1.0 g) were added to 15 mL of the MeOH:H_2O:AcAc solution and mixed for 30 s and then kept in ultrasound for 15 min. After new mixing, the samples were centrifuged at 3500 rpm for 10 min at 25 °C. The process of extraction was repeated with 10 mL of the solution and the extracts were combined and, then used for analyses after filtering (0.45 μm syringe filter).

Analyses of total phenolic compounds, total flavonoids, monomeric anthocyanins, and hydrophilic oxygen radical absorbance capacity tests (ORAC) were performed in the MeOH:H_2O:AcAc extract, according to methods previously described (Batista, da Silva et al., 2017). In order to identify and quantify the anthocyanins in SMF extract, we conducted the HPLC-DAD-ESI/MS analysis (liquid chromatography coupled with a diode array detector or mass spectrometry using the electrospray interface) as detailed in a previous study (Batista et al., 2017). All results were expressed in respective units per gram of fruit.

2.2. Animals and diet procedures

All *in vivo* experiments followed the ethical guidelines of the Brazilian Society of Laboratory Animal Science (SBCAL) and were approved by the Institutional Committee for Ethics in Animal Use (CEUA/IB/UNICAMP, protocol #3157-1).

Forty male Swiss mice (*Mus musculus,* 8-weeks old, 37.6 ± 3.5 g at the beginning of experiments) acquired from the Multidisciplinary Center of Biological Investigation (CEMIB/UNICAMP) were housed individually in cages under 22 ± 2 °C, 50–60% humidity and standard inverted 12/12 h dark/light cycles. During 10 weeks of experimentation, the animals had free access to water and the following semi-purified based-diets (AIN93-M) (Reeves, Nielsen, & Fahey, 1993): (i) a normal-fat diet (NF); (ii) normal-fat diet containing 5% (w/w) of *S. malaccense* fruit (NS); (iii) a high-fat diet (HF), in which 60% total calories were fat; and (iv) the high-fat diet containing 5% (w/w) of the *S. malaccense* fruit (HS) (Table 1). The 5% SMF added in the NS and HS diets provided to mice about 0.6 mg of total (poly)phenols and 14 mg of total soluble dietary fiber daily (Batista, da Silva et al., 2017) compounds that were supplemented in such diets (Table 1). The animals were divided into 4 groups in accordance with the diets NF, NS, HF or HS (n = 10/group). The diets were prepared tree different times (3 batches) along with the 10-weeks experimentation. Each diet was analyzed regarding its macronutrients and maintained frozen and protected from light until use. In order to control the nutritional quality of the diets, the mice received new food every 2 days, when they were served after defrosting. Food consumption was calculated considering the amount of food given subtracted by the leftovers in the containers and cage every 2 days. Bodyweight was measured weekly.

2.2.1. Cognitive test - Morris water maze (MWM)

At the 8th week of treatment, the spatial learning and memory based on hippocampal functions were assessed always at the same period, during the inverted dark cycle (between 11:00 a.m. and 12:30 p.m.) using an adaptation of the MWM test (Morris, 1984) described in a previous study and as follows (Batista et al., 2017). The maze consisted of a circular pool (120 cm in diameter, 50 cm high) filled with water (25 ± 1 °C) that was made opaque with non-toxic grey paint. The pool was divided into four quadrants. A circular plastic escape platform (9 cm in diameter) was placed in one quadrant into the pool approximately at 1 cm below the water surface. Mice were trained in MWM over five consecutive daily sessions, the acquisition phase. A trial was started when the animal was released from one of three randomly chosen start positions. The escape latency was recorded (maximum of 60 s) when the mice found and climbed onto the platform. Twenty-four hours after the last acquisition session (day 6), a probe trial was used to assess spatial retention of the location of the hidden platform. For this purpose, the platform was removed from the maze, and each mouse was allowed swimming freely to search for the platform for 60 s. During the probe test, the circular area surrounding the location where the platform was previously hidden was delimited three times larger and used as a counting zone to determine the times crossing the area. The time of swimming into the quadrant where the platform was previously hidden was also recorded.

2.2.2. Insulin resistance assessment

The glucose tolerance test (GTT) was performed injecting a D-glucose solution (2 g kg^{-1}) via intraperitoneal (i.p.) in the 6 h-fasted mice (n = 6 per group). Blood glucose was measured via tail vein at fasting, 30, 60, 90 and 120 min after the injection. The test was carried out in the 9th week of the experiment using a glucometer and respective test strips (FreeStyle Lite, Abbott, Alameda, CA, USA).

For the insulin tolerance test (ITT), a 0.9% saline solution containing 0.75 units kg^{-1} insulin (Novolin R, Novo Nordisk Bagsvaerd, DK) was i.p.-injected in the 6 h-fasted mice and blood glucose was measured via tail vein at fasting, 10, 20, 30, 45 and 60 min after the injection (n = 6 per group). The ITT was done in the 10th week of treatment using glucometer and test strips.

Soon before the euthanasia, the 6 h-fasting blood glucose level was measured via tail vein using glucometer plus test strips. Insulin levels were assessed in serum using a specific ELISA kit (Cat. #EZRMI-13K, Millipore, St. Charles, MO, USA).

2.2.3. Sampling

After the 10 weeks of the experiment, the animals were anesthetized (100 mg/kg ketamine chloride: 10 mg/kg xylazine chloride (Fortvale, Valinhos, SP, Brazil)) and euthanized in the dark cycle (between noon and 3:00 p.m.). The blood samples were obtained by cardiac puncture and collected in a serum separator gel tube.

After euthanasia, brains were quickly removed, washed in a 0.9% saline solution, weighed, frozen in liquid nitrogen and kept at −80 °C in ultra-freezer until use.

The hippocampus from left and right hemispheres were dissected by first removing the hindbrain and olfactory bulb and further detachment of them from the cerebral cortex. Due to the presence of ventricles between the midbrain and neocortex, the hippocampi could be easily separated as described previously (Batista et al., 2017).

Epididymal adipose tissue (EAT), retroperitoneal adipose tissue

Table 1
. Composition of experimental diets (g kg^{-1}) by mice groups.

Ingredient	NF	NS	HF	HS
Casein (83.95% protein)	141.75	141.75	141.75	141.75
Corn starch	464.56	441.27	264.25*	240.96
Maltodextrin	154.62	146.87	87.95*	80.20
Sucrose	99.76	94.76	56.74*	51.74
Soy oil	40.00	40.00	40.00	40.00
Lard	–	–	310.00	310.00
Cellulose	50.00	36.04**	50.00	36.04**
Mineral mix	35.00	35.00	35.00	35.00
Vitaminic mix	10.00	10.00	10.00	10.00
L-Cystine	1.80	1.80	1.80	1.80
Choline bitartrate	2.50	2.50	2.50	2.50
tert-Butylhydroquinone	0.008	0.008	0.008	0.008
S. malaccense fruit	–	50.00	–	50.00
Anthocyanins (mg kg^{-1})[a]		103.57		103.57
Flavonols (mg kg^{-1})[b]		3.91		3.91
Flavanols and procyanidins (mg kg^{-1})[b]		4.57		4.57
Soluble dietary fiber (g kg^{-1})[b]		2.81		2.81
Calories[a] (kcal kg^{-1})[c]	3786.18 ± 2.69	3773.90 ± 7.27	5464.62 ± 12.89	5426.25 ± 33.22

NF = group that received normal-fat diet; NS = group that received normal-fat diet containing 5% SMF; HF = high-fat diet group; HS = high-fat diet supplemented with 5% SMF.

* The carbohydrate ingredients were changed according to the addition of red-jambo fruit powder and/or lard content.

** The cellulose content was discounted according to the amount of insoluble dietary fibers of red-jambo pulp + peel (Batista, da Silva et al., 2017).

[a] Value calculated based on the current analysis presented in Sections 2.1 and 3.1.

[b] Value calculated based on a previous study (Batista, da Silva et al., 2017).

[c] Calories values were calculated considering the conversion factors of Atwater.

(RAT), and mesenteric adipose tissue (MAT) of mice were rapidly dissected, washed in 0.9% saline solution, dried on appropriate tissue paper and weighed.

2.2.4. Antioxidant status analyses

The measurements of thiobarbituric acid reactive substances (TBARS), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GRd), and superoxide dismutase (SOD) were performed in serum and brain frontal lobe homogenized in phosphate buffer (PB), pH 7.4 (approximately 100 mg mL^{-1}) (n = 6/group) as detailed in previous studies (Â. Batista et al., 2017; A. G. Batista et al., 2014). Brain frontal lobe has a close relation to prospective memory and correlates with hippocampal memory function (McFarland & Glisky, 2009; Poulose, Bielinski, Carey, Schauss, & Shukitt-Hale, 2017; Takahashi et al., 2007).

2.2.5. Western blotting

Western blotting was performed in hippocampi homogenate (n = 3–5 per group) from the same non-fasted animals used for antioxidant enzyme activity analyses (Section 2.2.4). Briefly, frozen hippocampi were homogenized in an extraction cocktail as described in a previous study (Batista et al., 2017). After electro transference, the membranes were prior incubated with 5% bovine serum albumin (BSA) to block non-specific antigenic sites followed by washing with TBS-T (0.1% Tris-buffered saline with 0.05% Tween 20, pH 7.4). Subsequently, the membranes were incubated with primary antibodies as follows: p-IRS-1 (Tyr 632)-R, IRS-1 (E-12), p-AKT 1/2/3 (Ser 473)-R, AKT 1/2/3 (H-136), p-GSK-3β (Ser 9), GSK-3β (H-76), p-tau (Thr 205), and tau (TAU-5) from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Then, the membranes were washed with TBS-T and incubated with the appropriate secondary antibody. The protein bands were visualized using an enhanced chemiluminescent substrate (Thermo Scientific, Waltham, MA, USA) and blot band densitometry was acquired using the NIH Image J 1.45s software (Wayne Rasband, NIH, Bethesda, MD, USA). All proteins were normalized against the corresponding values for β-actin (Sigma-Aldrich) endogenous control and the results were expressed as a ratio of p-proteins/total proteins in the case of the phosphorylated ones.

2.2.6. RNA extraction, quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted from EAT of non-fasted mice (n = 5/group) using a commercial acid phenol reagent, Trizol (Invitrogen, CA, USA). RNA integrity was confirmed by non-denaturing agarose gel electrophoresis. First-strand complementary DNA was synthesized using SuperScript III RT and random hexamer primers, as described in the manufacturer's protocol (Invitrogen). qPCR was run to determine the expression of IL-1β (Mm00434228_m1) and IL-6 (Mm00446190_m1) and TNF-α (Mm00443258_m1); all from Applied Biosystems, CA, USA. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (#4352339E; Applied Biosystems). The qPCR analysis of gene expression was carried out in an ABI Prism 7500 sequence detection system (Applied Biosystems). Each PCR contained 25 ng of reverse-transcribed RNA and was run according to the manufacturer's recommendations using the TaqMan PCR master mix. Real-time data were analyzed using the Sequence Detector System 1.7 (Applied Biosystems). Results were expressed as relative transcript amounts.

2.3. Statistics

All data were expressed as mean ± standard error (SEM). Data were analyzed using two-way ANOVA followed by Bonferroni post-test (p < 0.05). All tests were carried out using GraphPad Prism 5.0 software (GraphPad, Inc. La Jolla, CA, USA).

3. Results

3.1. Red-jambo fruit is rich in anthocyanins

Total phenolic, flavonoid and anthocyanin compounds were quantified in the freeze-dried red-jambo fruit, as presented in Table 2. The cyanidin-3-*O*-glucoside was the major anthocyanin found in red-jambo fruit (Table 2) as also confirmed by our laboratory (Batista, da Silva et al., 2017). However, other anthocyanins were identified, such as cyanidin-3,5-*O*-diglucoside and peonidin-3-*O*-glucoside (Table 2).

Antioxidant capacity assessed by ORAC showed hydrophilic

Table 2
Phenolic compounds and antioxidant capacity in *Syzygium malaccense* fruit.

Description	Units	Mean ± SD
Quantification of the phenolic compounds		
Total polyphenols	mg GAE 100 g^{-1}	229.61 ± 4.67
Total flavonoids	mg CE 100 g^{-1}	92.92 ± 3.24
Monomeric anthocyanins	mg C3G 100 g^{-1}	122.08 ± 3.55
Cyanidin-3-*O*-glucoside	mg 100 g^{-1}	207.13 ± 14.99
Identification of the anthocyanins		
Cyanidin-3-*O*-glucoside $[M+H]^+$ 449 = 287 *m/z*. (λ max (nm) = 280, 514), RT = 17.2 min		
Cyanidin-3,5-*O*-diglucoside $[M+H]^+$ = 611 = 449 and 287 *m/z*. (λ max (nm) = 270, 516), RT = 14.1 min		
Peonidin-3-*O*-glucoside $[M\ ]^+$ = 463, RT = 24.5 min		
Antioxidant capacity		
ORAC*	μmol TE g^{-1}	97.93 ± 6.05

SD = Standard Deviation; GAE = gallic acid equivalents; CE = catechin equivalents; C3G = cyanidin-3-*O*-glucoside equivalents; RT = Retention Time; ORAC = hydrophilic oxygen radical absorbance capacity test; TE = Trolox equivalents.

compounds contributed to around 100 μmol of Trolox equivalents per gram of the freeze-dried material used in the animal diets.

3.2. Red-jambo fruit powder supplementation did not affect body weight gain and brain weight

Among the groups, the HF group had the lowest value of food intake, but this group showed the highest calorie intake ($p < 0.01$), thus reflecting its higher body weight and weight gain in comparison to the NF group (Fig. 1). The bodyweight of HF animals was higher than in NF since the second week of the experiment until the 10th ($p < 0.01$). The supplementation of the normal and high-fat diets with SMF (NS and HS groups) did not cause a significant impact on the food/calorie intake nor in the body weight gain in comparison to their respective control group, as shown in Fig. 1. The group HS showed higher weight gain than the NS ($p < 0.001$).

Even though the body weight did not significantly change for the groups fed the SMF-containing diets, the EAT and RAT were smaller in the group HS when compared to HF ($p < 0.05$). The MAT weight was higher in HF in comparison to NF, and the HS showed the lower weight of MAT in comparison to the control HF ($p < 0.05$, Table 3).

The relative brain weight of the SMF-fed animals (NS and HS) was different between them but did not change in relation to their counterpart controls (NF and HF, respectively).

3.3. Red-jambo fruit intake improves peripheral insulin sensitivity

In both GTT and ITT tests, the HF group had a higher glycemic response when compared to the NF group (Fig. 2A–D). Although not statistically significant, the blood glucose level was lower in the HS group than in the HF group at the end of the blood glucose level curve (Fig. 2A), corroborating a trend towards the lower AUC in the HS group in comparison to HF (Fig. 2B). Blood glucose response in the ITT drove to similar results, showing significant insulin sensitivity in HS than in HF: lower glycemic curve and lower AUC values ($p < 0.01$, Fig. 2C and D). The fasting glucose level in the last day of experiments was also higher in HF in comparison to NF and HS groups ($p < 0.01$). No statistical differences were found in circulating insulin in response to high-fat diet feeding or SMF supplementation.

When qPCR was used to investigate inflammatory cytokine mRNA levels in the EAT, the HS group showed lower transcription for TNF-α relative to HF ($p < 0.01$; Fig. 2). No differences were found for both the IL-6 and IL-1β mRNA levels among the groups.

3.4. Red-jambo intake triggers serum and brain antioxidant defense

The antioxidant defenses in serum did not change with high-fat feeding, except for significant lower SOD activity in serum of HF mice in comparison to NF (Fig. 3). The lipid peroxidation in the serum of the HF was higher than in the NF group ($p < 0.05$). However, the addition of SMF into the normal-fat diet significantly increased serum GRd and CAT. When added to the high-fat diet, SMF revealed to improve serum GRd, GPx, SOD and CAT activities in the HS group, besides reducing lipid peroxidation in comparison to the HF group ($p < 0.05$).

The HF diet increased lipid peroxidation in the frontal lobe of the brain, as well as decreased the GSH concentration in comparison to NF (Fig. 3). In contrast and comparatively to HF, the HS diet prevented both lipid peroxidation values and enhanced the GSH values in the frontal lobe (Fig. 3A and B), hence evidencing the benefits of SMF supplementation to daily diet (Fig. 3).

The activities of GPx, SOD and CAT in the frontal brain of mice were reduced with high-fat feeding, as shown by the differences between NF and HF groups (Fig. 3). However, the GPx, SOD and CAT activities were higher in the brain of the HS group when compared to HF ($p < 0.01$, Fig. 3). There were no differences among the groups for GRd activity in the brain (Fig. 3).

3.5. Hippocampal phosphorylation of tau is minimized in red-jambo-fed animals

The insulin signaling pathway was studied in the hippocampi of mice, the region of the brain involved in learning and memory. Despite lower levels of phosphorylation of IRS in HF and slightly higher levels of p-IRS in the HS group, the values were not significantly different among the groups when two-way ANOVA was used (Fig. 4A). On the other hand, the phosphorylation of AKT in the hippocampi of the HS animals was higher than in the HF group, hence corroborating both the GSK3-β inactivation and the lower phosphorylation of tau in the HS group ($p < 0.01$, Fig. 4).

GSK3-β phosphorylation in serine 9 was reduced in the HF-group's hippocampus when compared to the NF counterpart, indicating higher activity of this kinase in the phosphorylating tau in the HF mice. These findings suggest that the addition of SMF to the high-fat diet may protect mice against AKT and GSK3-β activity-mediated hyperphosphorylation of tau ($p < 0.01$, Fig. 4).

3.6. Red-jambo fruit intake improved learning and memory

The Morris water maze cognitive test showed that mice fed HF-diet had worse performance in the acquisition phase, which was different from the observed in the normal-fat groups at sessions 4 and 5, and HS animals at session 3 (Fig. 5A). Thus, the supplementation with SMF improved learning on the acquisition phase, corroborating the better memory performance proven by the probe test (Fig. 5B and C). The finding could be expressed by the longer time spent swimming in the target quadrant and the crosses searching for the hidden platform recorded for the HS group in comparison to the HF groups ($p < 0.01$).

4. Discussion

In our study, we observed that 10 weeks-intake of SMF-supplemented high-fat diet may prevent oxidative stress, transcription of the pro-inflammatory cytokine, as well as peripheral insulin resistance and hippocampal tau phosphorylation in adult mice with no association to weight loss. This study shows that long-term ingestion of polyphenol/anthocyanin-rich diet at a period of life comprising young to middle age adulthood is beneficial to prevent cognitive impairment and development of AD' markers in individuals fed high-fat diet.

The previous report proved that SMF is rich in (poly)phenols and mainly anthocyanins like cyanidin 3-glucoside (Batista, da Silva et al.,

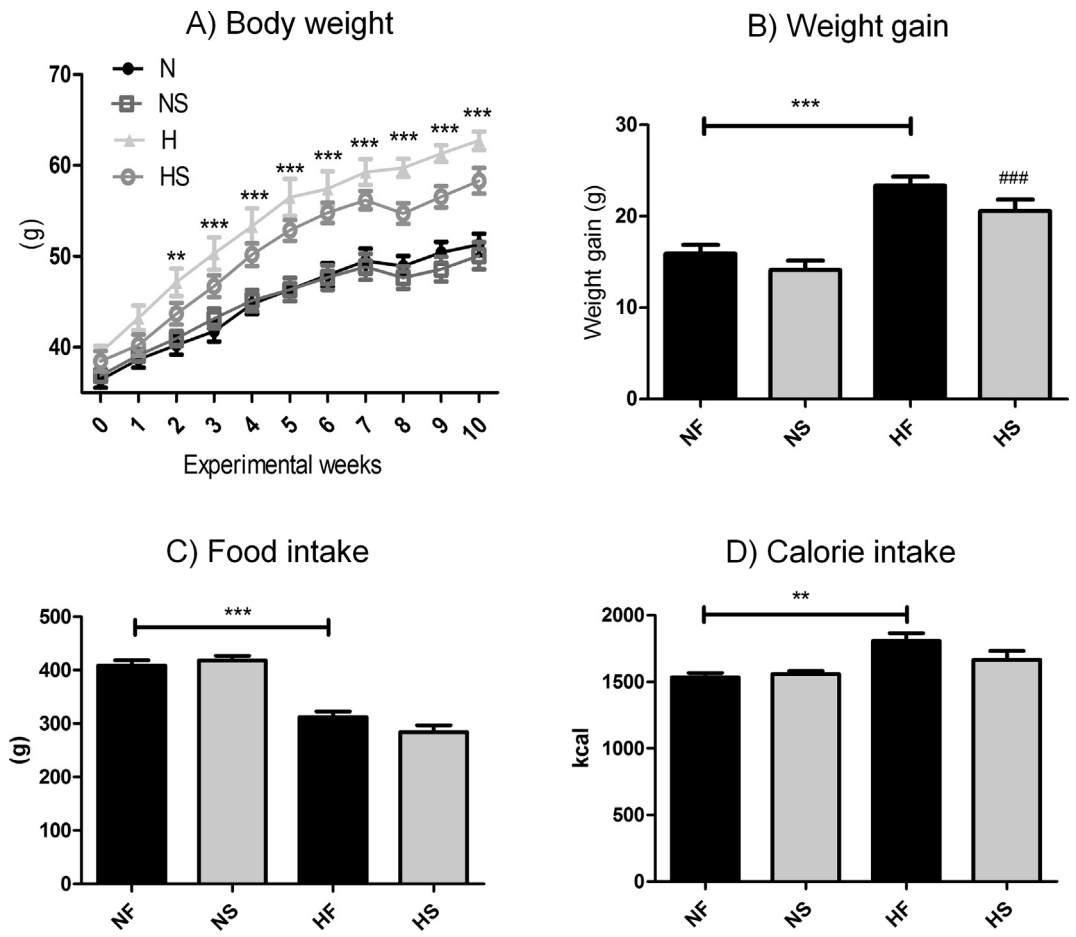

Fig. 1. Weight growing curves and feeding parameters. Data were assessed by two-way ANOVA and Bonferroni test (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$), $n = 10$/group. Data are expressed as mean ± SEM.

2017). Cyanidin 3-glucoside is one of the most common anthocyanins found in berries (Veberic, Slatnar, Bizjak, Stampar, & Mikulic-Petkovsek, 2015). The peel of SMF contains approximately 200 mg 100 g^{-1} of cyanidin 3-glucoside, claimed for its role in ameliorating obesity and diabetes metabolic markers (Tsuda et al., 2003). However, in the present study, the intake of the cyanidin-rich fruit was not able to prevent excessive weight gain linked to high-fat diet intake (Table 1).

Recent attention has been given to neuronal injuries caused by long-term intake of high-fat diets (Ledreux et al., 2016). Among such injuries, neuron loss, oxidative stress, insulin resistance, an increment of inflammatory markers and cognitive deficits can be cited, when aged rats and mice were fed high-fat diets during 5–6 months experimentation (Jeon et al., 2012; Ledreux et al., 2016). We demonstrated that a high-fat diet (35% w/w and 60% kcal of fats) promoted a reduction in brain weight and increased lipid peroxidation after 10 weeks of consumption (Batista et al., 2014). The present data showed that SMF addition to the high-fat diet did not prevent brain weight loss, but decreased lipid peroxidation, one of the markers of oxidative stress. Food components like (poly)phenols and other antioxidant compounds are metabolized and distributed in tissues, such as the brain, where they may also play a role neutralizing free-radicals formation (Batista et al., 2014).

The antioxidant enzymes are known to protect the cells against oxidative stress, a state that could trigger low-grade inflammation, insulin resistance and cell damage and/or death (Styskal, Van Remmen, Richardson, & Salmon, 2012). The biological antioxidant defenses were also increased in the serum and brain frontal lobe of HS animals, as it can be seen by the increased GSH content and the enhanced activities of the antioxidant enzymes GPx, SOD and CAT (Fig. 3). A possible beneficial effect related to the berry intake with these findings is the positive interference of (poly)phenols activating proteins in pathways responsible for the synthesis of antioxidant activities-related genes. For example, the nuclear erythroid 2-related factor 2 (NRF2) (Poulose et al., 2017), a transcription factor that up-regulates various cytoprotective genes, and insulin signaling cascade proteins (Dragano et al., 2013). Such compounds may also prevent the release of inflammatory

Table 3
Percent of the epididymal (EAT), retroperitoneal (RAT), mesenteric adipose tissue (MAT) and brain related to the body weight.

Tissue	NF	NS	HF	HS
EAT	4.18 ± 0.24	3.75 ± 0.24	5.28 ± 0.43*	3.51 ± 0.32**
RAT	1.72 ± 0.21	1.29 ± 0.12	2.69 ± 0.27*	1.49 ± 0.17**
MAT	1.95 ± 0.10	1.74 ± 0.40	2.84 ± 0.19**	1.98 ± 0.31*
Brain	0.614 ± 0.018	0.658 ± 0.040	0.532 ± 0.011**	0.538 ± 0.019

NF = group that received normal-fat diet; NS = group that received normal-fat diet containing 5% SMF; HF = high-fat diet group; HS = high-fat diet with 5% SMF. *Indicates $p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ according to Student's t test between groups (NF × HF and HF × HS) , $n = 10$/group.

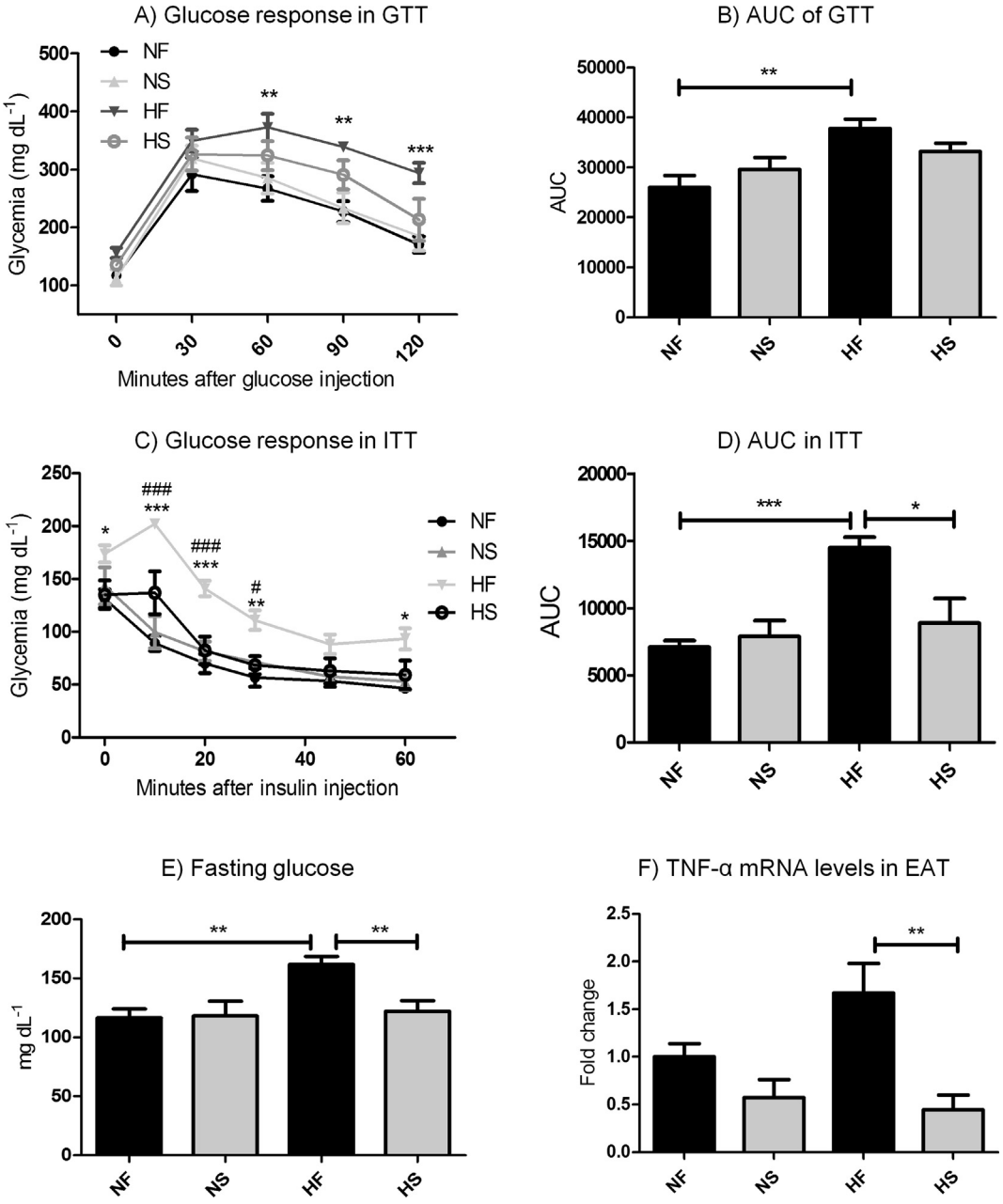

Fig. 2. Peripheral insulin resistance measurements, glucose and insulin levels in the mice. (*) Indicates differences between NF × HF and ($^{\#}$) HF × HS ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$) according to two-way ANOVA and Bonferroni test, n = 5–6/group. Data expressed as mean ± SEM.

cytokines via nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) inactivation (Dragano et al., 2013; Lee et al., 2014; Li et al., 2014).

A previous report has shown that the intake of anthocyanins and proanthocyanidins- rich berry provides a protection factor against insulin resistance in mice (Dragano et al., 2013). The findings showed that obese Swiss mice fed for 6 weeks a high-fat diet containing jaboticaba Brazilian berry showed higher peripheral insulin sensitivity via insulin tolerance test and activation of IRS-AKT-FoxO1 cascade (Dragano et al., 2013). A cross-sectional study had also shown that the intake of anthocyanin-rich foods was associated with lower insulin resistance, better economy on insulin release and lower inflammation levels in twin women (Jennings, Welch, Spector, Macgregor, & Cassidy, 2014). In the present study, besides enhanced peripheral insulin sensitivity observed with the supplementation of the high-fat diet with anthocyanin-rich SMF (HS) in comparison to HF, we also found improved hippocampal AKT and GSK3-β phosphorylation in the HS group. After 10-weeks SMF supplementation to mice fed high-fat diet, obese mice had the AKT-GSK3-β activated and the phosphorylation of tau (Thr 205) suppressed in their hippocampi, even without preventing weight gain. The same pattern was found previously when a high-fat diet supplemented with an isolated stilbene (resveratrol, 200 mg/kg) was given to young mice for 20 weeks (Jeon et al., 2012). We found no changes on insulin resistance or cognitive parameters when comparing NS to the NS group, likewise had been reported previously for mice fed high-fat diet and treated with resveratrol or jaboticaba berry fruit, respectively (Batista et al., 2017; Jeon et al., 2012). Interestingly, the findings suggest phenolic compounds are seemed not to boost cognition and insulin sensitivity in normal or healthy animals.

Together with β-amyloid, the phosphorylation of tau is one of the

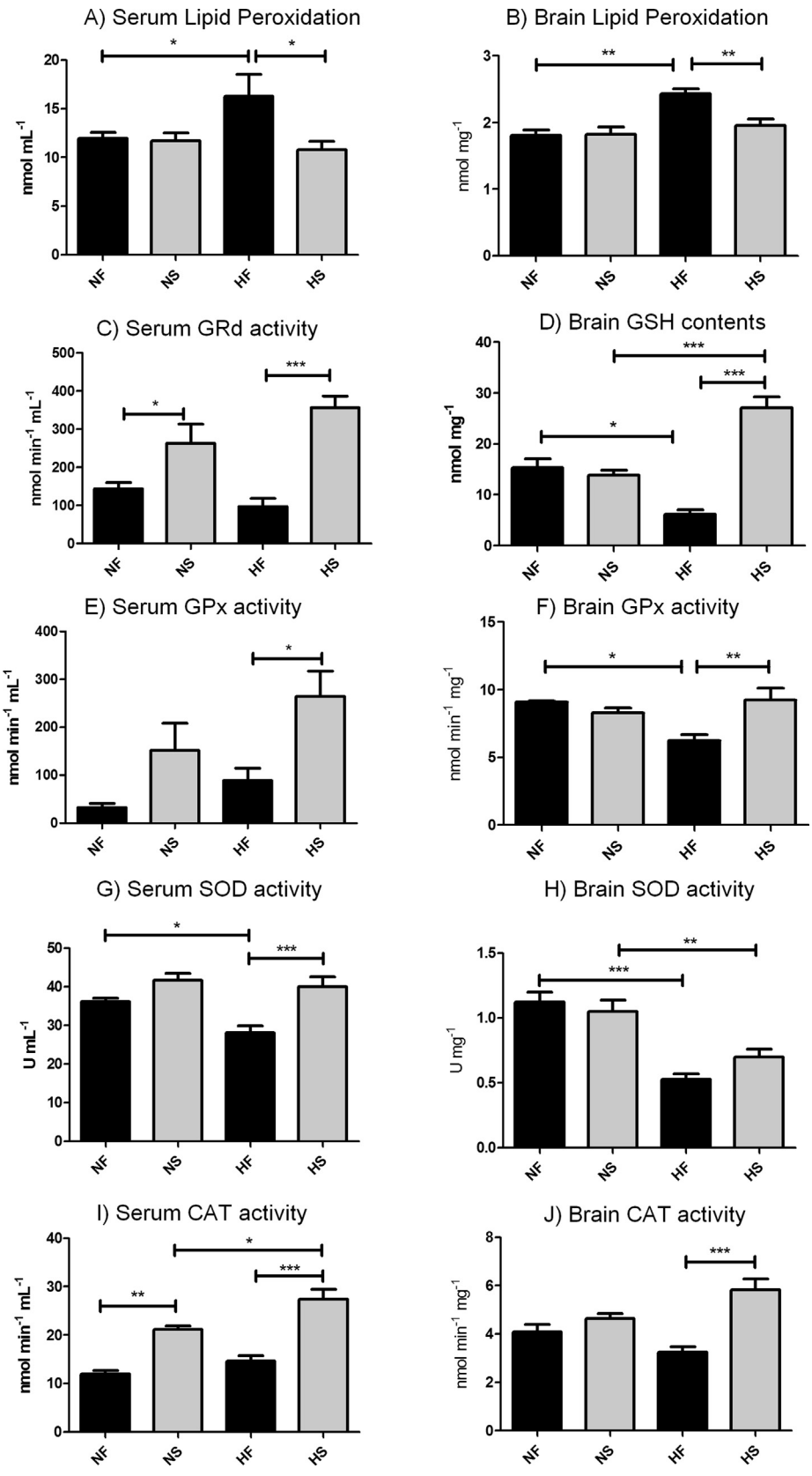

Fig. 3. *S. malaccense* fruit intake reduced lipid peroxidation and increased antioxidant enzyme activities in brain frontal lobe. (A) Lipid peroxidation or TBARS- thiobarbituric acid reactive substances; (B) GSH - reduced glutathione; (C) GPx - glutathione peroxidase, (D) GRd - glutathione reductase, (E) SOD - superoxide dismutase and (F) CAT - catalase. Data in (C) and (D) were expressed as nmol min^{-1} consumed NADPH mg protein and in (F) nmol min^{-1} formed formaldehyde mg protein. $^{*}p < 0.05$; $^{***}p < 0.01$; $^{***}p < 0.001$ indicate statistical differences according to two-way ANOVA and Bonferroni test, $n = 5$/group. Data expressed as mean ± SEM.

most investigated markers of AD (Ma et al., 2009). GSK3-β activating tau hyperphosphorylation via insulin signaling in the hippocampus may be a link between high-fat diet consumption and cognitive impairment (Jolivalt et al., 2008; Koga et al., 2014; Ledreux et al., 2016; Schubert et al., 2004). The present work suggests the benefits of SMF supplementation of high-fat diet-fed mice on preventing hippocampal tau phosphorylation and enhancement of memory as seen in the MWM test. A study has also demonstrated that animals fed high-fat based-diet

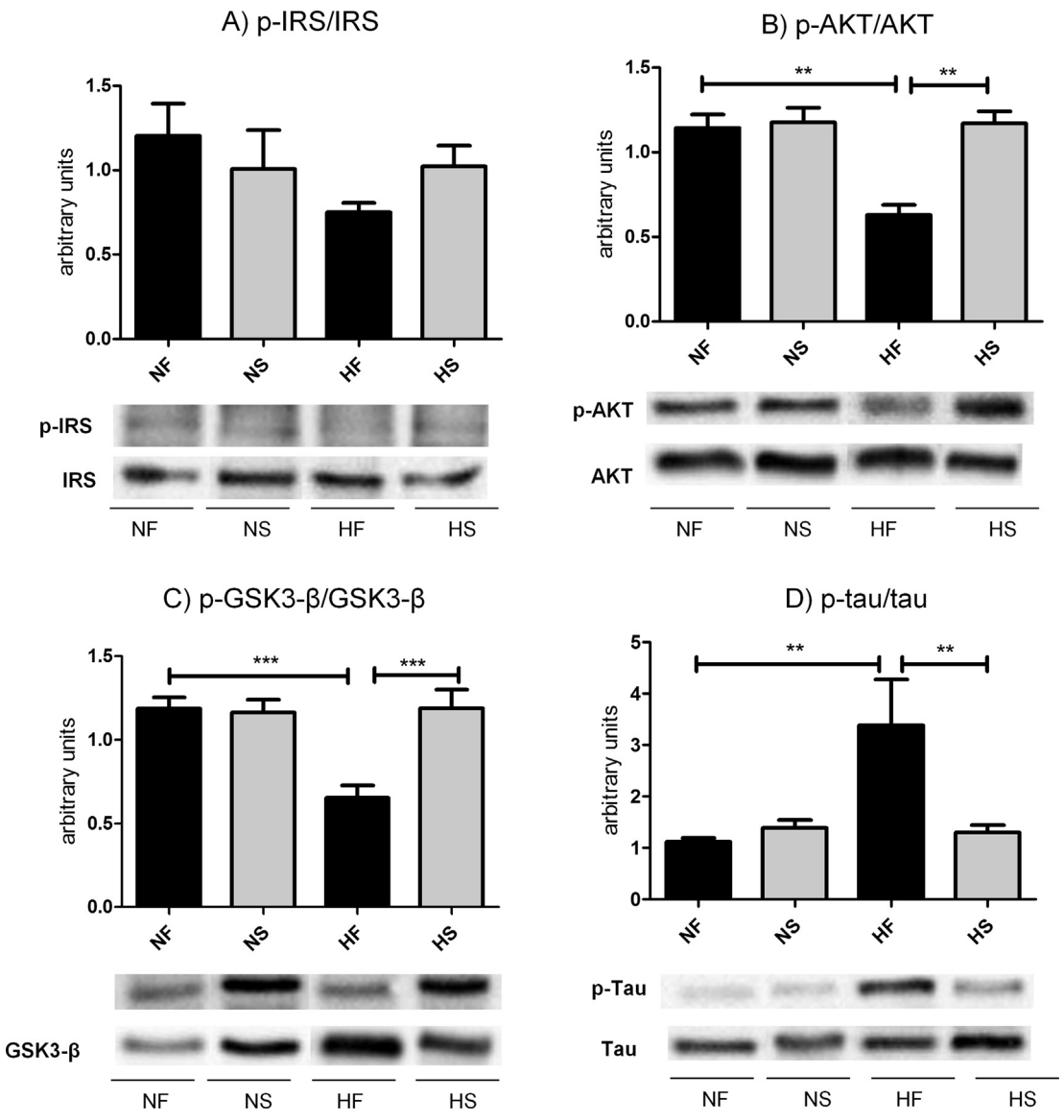

Fig. 4. *S. malaccense* intake prevents tau hyperphosphorylation via the hippocampal AKT-GSK3-β-Tau pathway. *$p < 0.05$; ***$p < 0.01$; ***$p < 0.001$ indicate statistical differences as assessed by two-way ANOVA and Bonferroni test, $n = 5$/group. Data expressed as mean ± SEM.

containing resveratrol, but not the supplementation of the normal-fat diet with the same compound, showed lower tau phosphorylation in the hippocampus and had both learning and memory improved (Jeon et al., 2012).

The underlying mechanisms for the berry fruit intake's effect on improvement in cognitive functions consist on indirect effects, like adjustments on inflammatory cytokines release (Poulose et al., 2012), and lower oxidative stress (Duffy et al., 2008; Papandreou et al., 2009), facts that are also supported by our findings. Moreover, other mechanisms might exist, since studies reported increased neurogenesis in

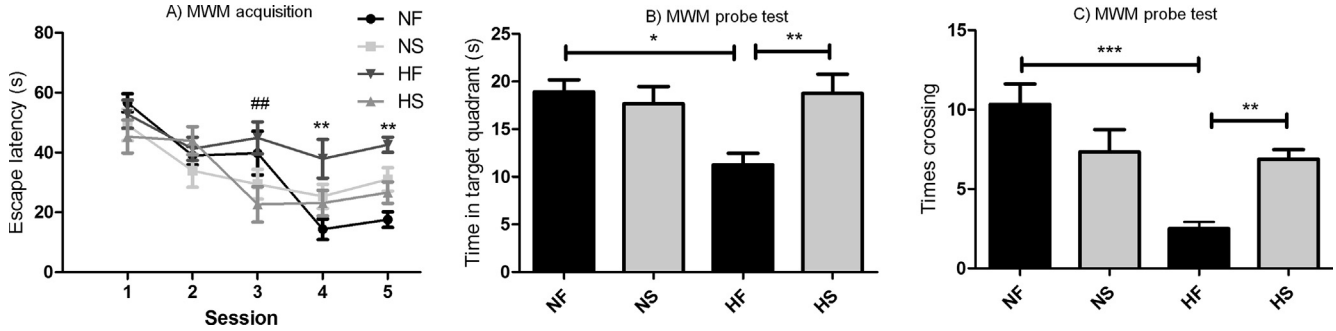

Fig. 5. *S. malaccense* fruit improves learning and memory assessed by the Morris Water Maze (MWM) test. A) (*) Indicates statistical differences between NF × HF groups, and ($^{\#}$)HF × HS according to two-way ANOVA and Bonferroni. B and C) Data were assessed by two-way ANOVA and Bonferroni test (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$), $n = 8$–10/group. Data expressed as mean ± SEM.

the hippocampus of aged mice and increases in neurotrophin expression in young and aged rats after berry consumption (Casadesus et al., 2004; Rendeiro et al., 2012).

Taken together, our results showed that the supplementation of anthocyanin-rich *S. malaccense* fruit to mice fed high-fat diet was able to improve the antioxidant defenses, peripheral and hippocampal lower phosphorylation of tau. The cognitive test corroborated the findings since the high-fat diet containing the peel and pulp fruit extract prevented the detrimental effects of the high-fat diets on learning and memory. We suggest that the nutritional introduction of such a supplement in the diet could be instrumental for preventing risk factors of cognitive impairment in an individual with high-fat diet consumption.

5. Ethics statements

This study was approved by the institutional Ethics Committee in Animal Use (CEUA UNICAMP, protocol #3157-1) and is in accordance to the CONCEA (National Council for Controlling the Animal Experimentation) and Brazilian Society of Science of Laboratory Animals (SBCAL) guidelines for ethical and welfare use of laboratory animals.

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CRediT authorship contribution statement

Ângela Giovana Batista: Conceptualization, Project administration, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Monique Culturato P. Mendonça:** Conceptualization, Investigation, Writing - review & editing. **Edilene Siqueira Soares:** Conceptualization, Investigation, Writing - review & editing. **Juliana Kelly da Silva-Maia:** Conceptualization, Investigation, Writing - review & editing. **Ana Paula Dionísio:** Investigation, Resources, Writing - review & editing. **Cesar R. Sartori:** Conceptualization, Resources, Writing - review & editing. **Maria Alice da Cruz-Höfling:** Conceptualization, Resources, Supervision, Writing - review & editing. **Mário Roberto Maróstica Júnior:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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